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Reviews in veterinary neurology and neurosurgery

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Editorial: Reviews in veterinary neurology and neurosurgery

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KEYWORDS

dyskinesia, epilepsy, hydrocephalus, meningoencephalitis, neuropathic pain

Editorial on the Research Topic
Reviews in veterinary neurology and neurosurgery

Introduction

In recent years, the generation of knowledge in the field of veterinary and comparative neurology and neurosurgery has grown exponentially (1). The impact, diversity, and potential future applications of this knowledge are showcased in the 13 papers featured in this Research Topic. These papers address a variety of problems relevant to practitioners of veterinary neurology from common and long-recognized but persistently challenging conditions, such as chronic pain, cognitive dysfunction, epilepsy, and non-infectious meningoencephalitis, to more recently described diseases that include craniocervical junctional anomalies and paroxysmal dyskinesias, along with highly technically focused and specific reviews of the neurosurgical management of congenital malformations.

Challenging the status quo: revising and refining diagnostic and management approaches for neuropathic pain, hemorrhagic CNS diseases and meningoencephalitis of unknown origin

Several articles in this Research Topic introduce new conceptual frameworks intended to facilitate the diagnosis, optimized management, or harmonized design of future studies of neurologic diseases. The contributions by Parker and Pedersen et al. address the complexities associated with the recognition and management of neuropathic pain in animals. These authors provide clinically relevant summaries of the form and function of nociceptive networks in health and disease and review advances in comparative pain research that collectively serve to improve the objective diagnosis of neuropathic pain, the rational selection of neuropathic pain modifying agents, and the development of new and effective analgesics. In their review of meningoencephalitis of unknown origin (MUO), Nessler and Tipold present a novel and holistic "polythetic" approach to the classification and treatment of MUO. Their proposal incorporates the heterogeneity and spectrum of clinical, neuroimaging, and immunopathologic manifestations of disease burden that may be present in each case in an effort to improve our understanding of the etiopathogenesis of MUO variants and to identify patient-specific therapeutic strategies. Santifort and Platt

review the reported causes, treatments and outcomes associated with conditions presenting with intraparenchymal hemorrhage in the brain or spinal cord in humans, dogs, and cats, and subsequently propose a novel and succinct veterinary classification schema for hemorrhagic encephalopathies and myelopathies that synthesizes comparative, etiologic, and neuroanatomic localization data.

Clinical applications of established and innovative technologies in veterinary neurology

The clinical utility of pervasive and emerging transformative technologies is illustrated in three papers included in this Research Topic. In their extensive review of paroxysmal dyskinesias (PD), Mandigers et al. highlight the value of phenotypic analyses of owner-acquired videos of paroxysmal events in conjunction with a focused historical probe and comprehensive clinical examination in the diagnosis and classification of canine PD. Robertson et al. provide an overview of the principles, promise, and limitations of a high throughput molecular analytical technique, Raman spectroscopy (RS), and demonstrate how RS-derived urine spectral fingerprinting can be used to identify and discriminate various etiologies of central nervous system disease in a rapid and economical manner. The article by Kim et al. describes the methodological development of a library of blood-based proteomic biomarkers of canine cognitive dysfunction, their validation against well-established mouse models of Alzheimer's and Parkinson's disease (2, 3), and the use of a machine learning analytical framework that revealed robust accuracies of the combination of retinol-binding protein 4 and NADPH oxidase 4 concentrations to clinically predict early neurobehavioral indicators of canine cognitive dysfunction.

Epilepsy: lessons from the past, current concepts, and promises for the future

Three articles in this Research Topic focus on epilepsy, emphasizing both the progress that has been made in the diagnosis and treatment of this disease and the current challenges that veterinarians face when managing epileptic animals. Gouveia et al. provide a historical narrative and comprehensive multispecies review of the oldest known antiseizure drug, bromide, which is infused with numerous evidence-based and practical pearls of wisdom related to the indications, efficacy, toxicity, and nuances of therapeutic monitoring associated with bromide therapy. Articles by Foss and Billhymer and Kadler et al. provide state-of-the-art reviews on the established and evolving uses of qualitative and quantitative brain magnetic resonance imaging techniques in the context of epilepsy and highlight how these techniques have advanced the field of human epileptology, as well as the limitations that remain in incorporating these techniques into routine use in veterinary practice.

Congenital malformations in companion animals

The final thematic area covered in this Research Topic relates to the diagnosis and treatment of congenital malformations affecting the brain, vertebral column, and spinal cord in dogs and cats. Schmidt and Farke provide a critical appraisal of the benefits, surgical techniques, and possible adverse events associated with ventriculoperitoneal shunting (VPS) for the treatment of internal hydrocephalus. This article includes excellent overviews of the diverse inventory of VPS instrumentation available for use, along with strategies for preventing and mitigating the range of complications that may be encountered in hydrocephalic patients treated with VPS. In their systematic review of the literature, Wess and Kneissl determine the prevalence of a spectrum of craniocervical junction abnormalities, such as occipital hypoplasia (OH), syringomyelia (SM) and atlanto-occipital overlap (AO) in dogs, and compare the frequency and potential clinical significance of these anomalies between dogs with and without a brachycephalic conformation. OH, SM, and AO were found to be significantly more likely to occur in small-breed, brachycephalic dogs, attributable clinical signs were infrequent in dogs with OH or AO that did not have contemporaneous SM, and only 1% of all dogs had concurrent imaging evidence of all three conditions. Roynard and Dewey summarize the currently available literature reporting on the neurosurgical management of lumbosacral meningomyelocele (MMC) in dogs, and contribute new clinical, diagnostic imaging, operative technical, and outcome data derived from the authors' experiences managing an additional nine dogs with MMC. Although the current body of literature reporting post-operative outcomes in dogs with MMC remains extremely limited, the work of these and other authors of recent studies suggests that early surgical intervention in dogs with MMC may result in improvement of pelvic limb neurologic function deficits and urinary and fecal incontinence in some dogs, and may prevent neurological deterioration associated with tethered cord syndrome (4).

The reviews included in this Research Topic provide a wealth of practical information on a wide range of subjects relevant to all levels of clinicians and researchers involved in the study or practice of veterinary neurology. To continue to generate rigorous evidence-based research and best practices for patient care, and to realize the potential of the tools and techniques covered in this Research Topic, it will be important for future studies to incorporate, critically evaluate, and subsequently refine the conceptual frameworks introduced here, and to apply and further validate these novel diagnostic technologies and therapeutic approaches in larger and more diverse animal populations.

Author contributions

JR: Writing – original draft, Writing – review & editing. AT: Writing – original draft, Writing – review & editing.

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Systematic minireview of the craniocervical junction in dogs with and without brachycephaly

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Objective: To identify, quantify and compare clinical and concurrent imaging findings of occipital hypoplasia (OH), syringomyelia (SM) and atlanto-occipital overlapping (AO) in dogs with or without brachycephaly.

Methods: A focused systematic search for literature was performed in the Web of Science™, PubMed and Google Scholar databases. Both authors screened and classified the identified articles using EndNote and appraised the articles using the Critical Appraisal Skills Program checklists. The main clinical and concurrent imaging features were extracted and evaluated for coexistence of OH, SM, AO, and other imaging findings.

Results: Thirty-one articles were included in this minireview. For articles focusing on descriptions of OH, SM and AO, 249 dogs had at least one of these conditions, and 3 of these 249 dogs (1%) had coexistence of all three conditions. For articles focusing on descriptions of the dogs, OH, SM, and AO were identified in 552/19/11/11, 574/2/0/6, and 100/0/0/0 small brachycephalic, small non-brachycephalic, large brachycephalic, and large non-brachycephalic breeds, respectively. For all small brachycephalic dogs, the percentages of affected animals were 40% for OH ($p = 0.01$), 42% for SM ($p < 0.01$) and 7% for AO ($p = 0.033$). The number of dogs having AO and clinical symptoms is low ($n = 5$).

Conclusion: OH, SM and AO are more likely to affect small dogs. AO might be limited to small brachycephalic breeds owing to the geometry of the craniocervical junction. Hence, AO alone might not lead to SM. In individual dogs, readers should carefully interpret the clinical relevance of OH or AO in the absence of SM.

KEYWORDS

craniocervical junction, occipital hypoplasia, atlantoaxial overlapping, syringohydromyelia, brachycephalic, toy breed dog

1 Introduction

Although the inability to breathe normally is considered the greatest obstacle in the welfare of small brachycephalic dogs (1), neurological, ophthalmological, dermatological, thermoregulatory, and gastrointestinal problems are also common in brachycephalic breeds (2, 3). When focusing on the craniocervical junction, four morphological alterations have been described. First, an unusual early closure of spheno-occipital synchondrosis at 8–12 months of age (6 months earlier than reported in non-brachycephalic breeds) causes the typical head shape (4). Second, occipital dysplasia

results in either a slightly increased dorsal notch or an unusually enlarged foramen magnum. Other names for occipital dysplasia are caudal occipital malformation syndrome and occipito-atlantoaxial malformation (5–7). Absence or shortening of parts of the occipital bone has also been described as incomplete ossification (8, 9). Third, incomplete ossification of the atlas has been described as predisposing to atlantoaxial subluxation (10, 11). Overall, changes in the conformation of bony structures in the craniocervical junction are associated with individual differences in head and neck movements (12). Fourth, occipital hypoplasia describes the shortening of the basilar part of the occipital bone, causing a reduced volume in the caudal fossa. This can lead to overcrowding and subsequently to herniation of cerebellar matter through the foramen magnum, resembling Chiari-like malformation in humans. In addition to congenital abnormalities, a frequently described acquired abnormality in this region is syringomyelia (SM), which is characterized by fluid-filled cavities in the spinal cord parenchyma. While the exact pathophysiology of SM is unknown, it is related to an outflow obstruction of cerebrospinal fluid at the level of the foramen magnum and is described secondary to OH. Clinical signs may be like those of Chiari-like malformation or may be clinically silent (13–15). Atlanto-occipital overlapping (AO) has been described as non-traumatic overlapping of the occipital bone and neural arch of the atlas (7, 16–18). Imaging findings include diminished distance between the dorsal lamina of the atlas and the supraoccipital bone, with the dorsal lamina of the atlas located either immediately caudal to the foramen magnum or within it (16, 18, 19). AO has been implicated in the development of neuropathic pain and neurological dysfunctions such as ataxia (20).

The main objective of this systematic review was to identify, quantify and compare concurrent major imaging findings of OH, SM and AO in dogs with or without brachycephaly. The specific aims were to document the prevalence of SM with OH or AO in brachycephalic and small breed dogs compared with non-brachycephalic and large breed dogs, to list corresponding reported clinical symptoms, and to reevaluate the clinical relevance of AO in small brachycephalic dogs.

2 Materials and methods (search)

This review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (21). A systematic search for literature concerning the craniocervical junction was performed in the Web of Science™, PubMed and Google Scholar databases. The following keywords were searched: "occipital hypoplasia" OR "occipital dysplasia" OR "foramen magnum dysplasia" OR "incomplete ossification" OR "atlantoaxial overlapping" OR "syringohydromyelia" OR "syringomyelia" OR "Chiari-like malformation" AND "dog." Original articles, systematic reviews and meta-analyses were retrieved. The identified articles were imported to EndNote 21 [Clarivate Analytics (US) LLC, London, UK] and classified by both authors. Reasons for exclusions were duplicates or the article had a different focus, an undefinable population, or an impossible classification of corresponding imaging findings. Retrieved articles were appraised using the Critical Appraisal Skills Program (CASP) checklists for the quality and importance of their concepts, facts, and conclusions. To follow the aims of the study, the focus of review was (a) documentation of dogs having at least two reviewed imaging signs (including OH, SM, or AO), or (b) listing reported clinical symptoms being attributable to the reviewed imaging signs.

3 Results

Initial literature search found 1,226 articles in Web of Science™, 232 in PubMed and 9 in Google Scholar, which were narrowed down to 103 analyzed for article relevance depending on both title and abstract. The publication year ranged from 1993 to 2023. The full detail of the article selection process is documented in Table 1. Thirty-one articles including 1,484 dogs satisfied the inclusion criteria.

3.1 Prevalence of OH, SM, and AO

The following small brachycephalic breeds were reported: Affenpinscher, Bichon Frise, Boston Terrier, Brussels Griffon, Cavalier King Charles Spaniel, Chihuahua, Japanese Chin, Maltese, Papillon, Pekingese, Pomeranian, Pug, Shih Tzu, Toy Poodle, and Yorkshire Terrier. Small non-brachycephalic breeds included Cocker Spaniel, Jack Russel Terrier, Miniature Dachshund, and West Highland White Terrier. Large brachycephalic breeds included Boxer and Staffordshire Bullterrier. Large non-brachycephalic breeds included Bull Terrier, Border Collie, Golden Retriever, Labrador Retriever, and Springer Spaniel.

Table 2 displays the breed group distributions for OH, SM, and AO. For articles focusing on descriptions of OH, SM, and AO, 249 dogs had at least one of these conditions, and 3 of these 249 dogs (1%) had coexistence of all three conditions (Supplementary Table S1). All 249 dogs were small brachycephalic dogs. The other combinations were distributed as follows: OH occurred in 24% ($n = 59$), SM in 4% ($n = 9$), AO in 7% ($n = 18$), OH and SM in 61% ($n = 153$), and SM and AO in 3% ($n = 7$). No dogs were reported to have coexisting OH and AO.

Because reports varied in the level of detail of the information, such as a condition not being found or not investigated, individual dogs were further classified into subgroups. OH and SM were concurrently reported in 217 dogs, with 156 (72%) showing imaging features of both entities, 59 (27%) showing only OH, and 2 (1%) showing only SM. Six dogs had OH and AO, with 3 (50%) showing either imaging signs of both or AO only. Thirty-seven dogs had SM and AO, with 10 (27%) showing both, 9 showing SM only (24%), and 18 showing AO only (49%). Six dogs had all three imaging findings together; 3 (50%) showed concurrent imaging findings of all three, 2 showed SM and AO (33%) and 1 (17%) showed only AO.

For all 1,484 dogs, we identified 1,275 individuals showing OH, SM, and AO in 552/19/11/11, 574/2/0/6, and 100/0/0/0 small brachycephalic, small non-brachycephalic, large brachycephalic, and large non-brachycephalic breeds, respectively (Table 2). Of the small brachycephalic dogs, the percentages of affected animals were 40% for OH ($p < 0.01$), 42% for SM ($p < 0.01$) and 7% for AO ($p = 0.033$).

Abbreviations: AO, Atlanto-occipital overlapping; SM, Syringomyelia; OH, Occipital hypoplasia.

TABLE 1 Article selection process and final postulation.

Identification	Web of science™ (n = 1,226)	PubMed (n = 232)	Google scholar (n = 9)	"Occipital hypoplasia" OR "occipital dysplasia" OR "foramen magnum dysplasia" OR "incomplete ossification" OR "atlantoaxial overlapping" OR "syringohydromyelia" OR "syringomyelia" OR "Chiari-like malformation" AND "dog"
	Total (n = 103)			Full-text articles excluded by duplicity (n = 25)
Screening	Articles included for title and abstract screening (n = 88)			Full-text articles excluded for the following reasons (n = 57): a Different focus b Undefinable population c Impaired correlation of imaging findings
Eligibility	Articles selected for full-text analysis (n = 31)			Full-text articles included with reasons based on CASP (n = 31)
Included	Articles included for qualitative analysis in this systematic minireview (n = 31)			Conclusion a) Only 3/249 dogs (1%) exhibited coexistence of OH, SM and AO. b) OH occurrence was reported in all dog groups, whereas SM was limited to small dogs, and AO was limited to small brachycephalic dogs. c) Five dogs had AO and clinical symptoms. AO could be a normal variation in small brachycephalic dogs. In the absence of SM, its clinical relevance should be carefully interpreted.
	Discussion			

TABLE 2 Coexistence of occipital hypoplasia (OH), syringomyelia (SM) and atlanto-occipital overlapping (AO) in dogs reported between 1993 and 2023.

Group	OH	SM	AO	Total
Brachycephalic small breed	552	574	100	1,226
Non-brachycephalic small breed	19	2	0	21
Brachycephalic large breed	11	0	0	11
Non-brachycephalic large breed	11	6	0	17
Total	593	582	100	1,275

Four studies of AO concurrently reported the following imaging findings: an abnormally large foramen magnum, cerebellar involvement, medullary kinking, atlantoaxial instability, dens abnormalities, and syringohydromyelia (16, 17, 19). In the 100 dogs, reported to be affected with AO, additional imaging findings described were medullary kinking (n = 95), atlantoaxial subluxation/instability (n = 67), occipital dysplasia (n = 67) hydrocephalus (n = 42), ventriculomegaly (n = 24), compressive lesions of the spinal cord (n = 39), arachnoid diverticula (n = 5), intervertebral disk protrusion/extrusion (n = 2), dens hypoplasia (n = 1), and central canal dilatation (n = 15).

3.2 Reported clinical symptoms

Twenty-three studies reported clinical symptoms. Thirty-eight clinical symptoms were described, including hyperesthesia/pain (n = 95), gait abnormalities (n = 60), decreased postural reactions (n = 33), neck and/or shoulder scratching (n = 35), proprioceptive deficits (n = 24), seizures (n = 22), decreased head reflexes (n = 21), and non-specific neurologic symptoms (n = 31). Other symptoms were mentioned in lower frequencies (n = ≤10) or only mentioned but not quantified. Supplementary Table S2 summarizes most frequently reported clinical symptoms in dogs affected with OH, SM, or AO. A total of 132 reported clinical symptoms were attributable to OH, SM, or AO. The table documents that the number of dogs having AO and clinical symptoms is low (n = 5).

4 Discussion

4.1 Principal findings

In this minireview, 249 dogs were identified as having OH, SM or AO, and all were small and brachycephalic (Supplementary Table S1). OH, SM and AO coexisted in 3/249 dogs (1%) (6, 22, 23). OH and SM were concurrent imaging findings in 153 dogs (61%). OH (Chiari-like malformation) is known to be associated with SM in dogs, where the skull is too small for the brain (24, 25). Dogs affected, most reported Cavalier King Charles Spaniels, show neck pain, scratching at the neck or shoulder, facial rubbing behavior, and vocalization among other clinical signs (26). Surgical decompression is indicated in severe cases (syrinx ≥ 3 mm diameter on transverse T2 MR images) and resulted, combined with medical therapy, in long-term improvement in most patients (27). Eighteen dogs (7%) exhibited AO as a single imaging finding. AO might be limited to small brachycephalic dog breeds owing to the typical head shape and geometry of the craniocervical junction. The short cranial base together with the caudodorsally orientated dome-shaped head (13) and consecutively cranioconvex-shaped foramen magnum lead to a geometrically well-covered atlas that might be wrongly interpreted on a median plane to be in the foramen magnum. Other than congenital causes (4), the discrepancy between the volume (or pressure) of the soft tissues and bones may lead to pressure atrophy of the occipital bone (resulting in OH or occipital dysplasia) and pressure atrophy of the dorsal arch of the atlas (paper-thin, no medullary canal) (28). The bone interacts with variable pressure conditions and

reacts to them, which is described by the Wolff transformation law (29). The functional adaptation of the morphology of the head (dorsorotation of maxillary incisors, obstruction of the nasal cavity) (30) and atlas (highly oval-shaped and missing medullary cavity in the dorsal arch) (28) could also be a consequence of this local pressure increase.

4.2 Reevaluation of AO: is AO a key or a buried finding?

In this minireview, 38 clinical symptoms were described. Key (or major) findings significantly affect the odds of a specific diagnosis (31). Since 2009 (20), AO has been reported to be a key finding that influences the clinical signs and therapeutic outcome of dogs. Hence, surgical management is recommended (7). To date, AO has been surgically stabilized in two dogs; both were diagnosed with AO, but corresponding images documented coexisting severe atlantoaxial instability (32) or herniated cerebellar matter (19). A study of 41 dogs diagnosed with atlantoaxial instability and treated revealed that AO was a coexisting condition in 12 dogs. All dogs were surgically treated; however, presence or absence of AO did not affect the outcome (33). Conversely, buried findings are incidental (34) and not clinically significant. Apart from the original description, we did not identify further evidence that AO by itself is corresponding to clinical signs. However, in seven reported dogs AO and SM coexist (35, 36). Hence, whether AO represents normal variation in small brachycephalic dogs or is a key finding responsible for clinical signs and needs to be treated is unclear. Prospective observational clinical studies are needed to clarify the clinical relevance of AO.

4.3 Limitations

Limitations of this systematic review included the large variation of head morphologies among dog breeds and difficulties in correlating reported imaging findings and symptoms to individual dogs.

5 Conclusion

OH, SM and AO are more likely to affect small brachycephalic dogs than other types of dogs, possibly owing to the geometry of the craniocervical junction. Problems might arise from increased dorsorotation, which is associated with respiratory tract malformations (37) but also affects the craniocervical junction. Hence, AO alone might not lead to SM. We recommend classifying AO as a normal variation in small brachycephalic dogs rather than as a pathological finding that needs to be treated. In individual dogs, readers should carefully interpret the clinical relevance of OH or AO in the absence of SM.

Author contributions

LW: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. SK: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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Supplementary material

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Bromide: the good, the bad, and the ugly of the oldest antiseizure medication

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Bromide is the first effective antiseizure medication used in human medicine since the XIX century. Initially met with skepticism, bromide quickly gained enthusiasm within the medical field until being largely replaced by newer antiseizure medications with significantly fewer adverse effects in people. In veterinary medicine, bromide continues to be used in the management of epileptic patients for over 30 years, yet adverse effects can impact owners and patients alike. We sought to provide the general practitioner and veterinary neurologist with insightful information on both the positive and negative attributes of bromide, explore factors that may influence its desirability as an antiseizure medication in specific veterinary cases and elucidate its current role in modern epilepsy treatment for veterinary patients. It's also our endeavor to discuss the current use as an alternative or add-on with other known antiseizure medications and potential future studies that might enhance our understanding and use of this medication.

KEYWORDS

epilepsy, seizure, canine, adverse effects, salt, refractory epilepsy, ASM

1 Introduction

Epilepsy is the term used to describe a brain disease that leads to a persistent predisposition for the generation of epileptic seizures, with this term being applied to patients that experience at least two unprovoked epileptic seizures separated from more than 24 h (1).

A systematic review and metanalysis by Fiest et al. (2) identified a point prevalence of active epilepsy of 6.38 per 1,000 people.

In canine patients, epilepsy was found to be the most prevalent chronic neurological disorder (3, 4). Although the exact point prevalence is not known, this was estimated to be between 0.6 and 0.75% in the general dog population (1). Epilepsy can also occur in cats. O'Neil et al. (5) reported epilepsy with a one-year period prevalence of 0.04% for patients presenting to primary veterinary care practices in the United Kingdom.

Idiopathic epilepsy (IE), which can be further subclassified into genetic epilepsy, suspected genetic epilepsy or IE of unknown cause, is the most common cause responsible for seizures in dogs (1, 4). Seizures can also be a consequence of intracranial disease (structural epilepsy), metabolic disorders or intoxication (reactive seizures) (6).

Treatment of patients suffering from epilepsy is recommended according to the patient's seizure frequency, severity of ictal or post ictal phase, owner's beliefs, and lifestyle (7). Adequate seizure control can improve the quality of life for both canine patients and their owners (8).

In addition to this, seizure frequency might increase overtime in untreated patients suffering from idiopathic epilepsy, emphasizing another possible advantage of initiating treatment in a timely fashion (Table 1) (7, 10).

The use of antiseizure medications (ASMs) is the current mainstay for the treatment of epilepsy.

Phenobarbital is the oldest and most commonly used ASM in veterinary patients (11). It is well tolerated, has a widespread availability and low cost, making it the primary treatment choice in canine epilepsy (10, 11). In addition to phenobarbital, imepitoin and potassium bromide are the only approved ASM for the treatment of canine epilepsy in Europe (11). In the United States potassium bromide (KBroVet-CA1) and phenobarbital (Fidoquel-CA1) are currently the only two medications that received conditional approval by the Food and Drug Administration (FDA) in January 2021 and September 2023, respectively (9, 12). The use of primidone, levetiracetam, zonisamide, felbamate, topiramate, gabapentin, pregabalin is also described, although the levels of evidence supporting their efficacy vary considerably (13).

Bromide is the name given to a negatively charged bromine ion (14). It is usually administered associated to potassium, in the form of potassium bromide (KBr) and can be used in monotherapy or as an add-on ASM (15).

Bromide has now been successfully used as an ASM in the treatment of canine epilepsy for more than three decades (14, 16).

The aim of this paper is to give a historical perspective of the use of bromide in the treatment of epilepsy in veterinary and human medicine, review its mechanisms of action, pharmacokinetics, currently reported efficacy, known adverse effects and tolerability, possible drug interactions, current indications for serum level monitoring and have an outlook on how it currently compares with other frequently used ASMs. The present research gaps and potential future developments in the use of this medication are also reviewed and discussed.

The information presented in this review aims to enhance the understanding of primary practitioners and veterinary neurologists regarding the current insights into the use of bromide in epileptic patients. It seeks to raise awareness about both the positive and negative aspects of this substance, empowering clinicians to make more informed decisions when utilizing this well-established ASM.

2 Historical perspective

In human medicine, bromide was recognized as an effective antiepileptic medication in 1861, after being previously used in 1857 by Sir Charles Locock, an obstetrician for Queen Victoria, to treat young woman with "hysterical" epilepsy and "catamenial seizures," connected with the menstrual period (17–20).

Bromide was previously better known for its efficacy in improving clinical signs associated with nymphomania or priapism, and its newly discovered antiseizure effects only got more attention at a later stage, in the mid-1860s (21).

Despite the lack of any randomized studies at that time, the available clinical data is considered enough to name bromide as the first effective ASM, marking the beginning of the modern treatment of human epilepsy (19).

After the initial use of potassium bromide, other different formulations were also tried, including its association to sodium or ammonium. Neither of the attempted alternatives revealed increased efficacy in the control of seizures or decreased adverse effects, which became one of the main drawbacks of bromide therapy (21). Reckless use of this medication with excessively high doses and lack of adequate follow-up were also one of the suspected reasons behind the development of unacceptable mental dullness and apathy in people (21). Adverse effects affecting the patient's skin also became well recognized, with the development of cutaneous eruptions (bromoderma) that added significant morbidity to human patients treated with this medication (22).

Bromide continued to be the only effective ASM used in human medicine until 1912, when the antiseizure properties of phenobarbital were discovered (18). When compared to bromide, the decreased sedative effects associated with phenobarbital and newer molecules made them to gradually replace and be preferably used (18, 23).

Despite having a currently more limited role in human epilepsy, bromide can still be considered and have an important role in the treatment of refractory pediatric epilepsy (24, 25).

Schwartz-Porsche and Jürgens (26) described the use of potassium bromide as an effective add-on therapy (in addition to phenobarbital and/or primidone) to treat canine patients with refractory epilepsy for the first time in veterinary medicine in 1991. In this study, bromide led to an improvement in seizure control in 11 of the 22 dogs that composed the cohort, 4 become seizure free and 7 had a reduction of seizure frequency of at least 50% (26).

Podell and Fenner (23) and later Trepanier et al. (27) provided further evidence for the usefulness of bromide in the treatment of canine epilepsy, with studies revealing an overall efficacy of 83 and 72% in reducing seizure frequency and exponentially increasing the interest of this substance in veterinary epilepsy.

Following its initial use as an add-on therapy in association with phenobarbital, reports on the use of bromide as an effective alternative first-line antiepileptic also became available (16, 28).

TABLE 1 International veterinary epilepsy task force (IVETF) current indications to recommend maintenance ASM treatment (9).

Interictal period equal or inferior to 6 months
Status epilepticus or cluster seizures
Severe postictal signs (e.g., aggression) or lasting longer than 24 h
Increasing seizure frequency and/or duration and/or severity over 3 interictal periods

3 Mechanism of action and pharmacokinetics

Bromide's mechanism of action seems related to its preferential movement across neuronal chloride channels. This facilitates its intracellular accumulation in the neurons and increased gamma-aminobutyric acid (GABA) inhibition due to hyperpolarization of the membrane (29–32). Medications like barbiturates (e.g., phenobarbital), which boost chloride conductance through GABA-ergic activity, may work together with bromide to elevate the seizure threshold (29).

Although an active transport system in the choroid plexus can remove accumulation of bromide from the CSF and CNS, this system can be overwhelmed if administration of bromide is high enough (33).

GABA release, binding, transport or metabolism, however, does not appear to be affected by acute or chronic exposure to bromide (34).

After oral administration, bromide is easily absorbed in the gastrointestinal tract and has an estimated mean bioavailability of 46% (35), and maximal concentration in the cerebrospinal fluid occurs in about 2 h (36). In contrast with other antiseizure medications, bromide does not go through hepatic biotransformation and has no hepatotoxic effects (29). Canine thyroid function also does not seem to become affected by the chronic use of bromide (37, 38).

The unchanged halide of bromide is eliminated in the urine after being filtered from the bloodstream at the level of the glomerulus (39). After glomerular filtration, bromide suffers extensive tubular reabsorption leading to its long elimination half-life (39). Elimination half-life is variable and reported between 15 (40) and 46 days in dogs (35), approximately 11 days in cats (41) and 12 days in humans (42).

As with any medication, a steady-state serum concentration is reached after about four to five eliminations half-lives of regular administration (43). For this reason, in canine patients, bromide steady-state concentrations are expected to take 2–3 months to achieve (44). Despite this, therapeutic serum concentrations can still be achieved before reaching steady-state concentrations, what can justify the administration of initial loading doses in selected patients (35).

Chloride and bromide compete for tubular reabsorption, with bromide being naturally more easily reabsorbed and chloride more easily excreted (39, 45). An increase in chloride intake can enhance bromide renal elimination (35, 39, 46). Changes in chloride intake can occur with changes in dietary salt (sodium chloride) content.

Mercurial diuretics can increase bromide elimination, suggesting that bromide reabsorption might occur via the chloride channels in the thick ascending limb of Henle (47, 48). Loop diuretics like furosemide might also increase bromide elimination by blocking chloride and bromide reabsorption (49, 50). Osmotic diuretics have no impact on bromide elimination (39).

4 Dosage and administration routes

Bromide is typically administered orally in the form of the salt potassium bromide (KBr) (51). Sodium bromide (NaBr) constitutes an alternative, less common formulation that can also be used in veterinary patients. Currently, the use of NaBr is limited by the absence of commercially available formulations. Dosage must be adapted accordingly to the salt that is being used. The dose for NaBr is approximately 15% less of that of KBr, since potassium has a higher molecular weight than sodium, making 1 g of NaBr to contain more bromide than 1 g of KBr (28, 44, 51). Sodium bromide can be particularly useful in dogs with disease where administration of potassium might be undesirable (e.g., hypoadrenocorticism) but is contraindicated in case of hypertension, congestive heart failure or hepatic disease (28).

In canine patients, potassium bromide dosage as add-on therapy (e.g., in association with phenobarbital) is 20–40 mg/kg/day (11, 26, 28, 44, 51). Doses between 30 and 40 mg/kg/day can be adequate when using bromide in monotherapy (11, 51, 52). Although Trepanier (28) reported doses between 50 and 80 mg/kg/day to be possibly needed in some patients treated with bromide alone, the authors believe these doses are most likely excessively high and not indicated in most patients. Due to bromide's long elimination half-life, dosing can be performed only once daily, what might help increasing owner's compliance (44).

A dosage of 30 mg/kg/day was suggested for use in cats (41), but currently there is a weak level of evidence regarding the efficacy and safety of this medication in feline patients (53). The limited number of studies assessing bromide use in feline patients is likely related to the frequent adverse effects associated with its use in this species (as discussed in another section of this manuscript).

In horses, Raidal and Edwards (54) described the use of a loading dose of 120 mg/kg/day during a 5-day period and maintenance doses of 90–100 mg/kg of potassium bromide administered once daily. This protocol appears to lead to serum bromide concentrations that are associated with clinical efficacy for seizure control in other species.

Given bromide's extended elimination half-life, a loading dose can be administered to reach the desired therapeutic serum concentrations levels faster in selected patients (14, 44, 51). Different loading dose protocols were previously described (14, 51, 55).

A loading dose of 125 mg/kg, divided into two daily administrations over 5 days, has been previously suggested. This approach results in a total loading dose of 625 mg/kg by the end of the 5-day period (51). After the end of the loading period, dogs should continue receiving the normal maintenance bromide dose.

Alternatively, in an emergency (e.g., patients in status epilepticus) the loading dose of 600 mg/kg can be administered quicker, over a 24-h period, in multiple 100 mg/kg dosages given 4 h apart (55). Given that loading doses may carry a higher risk of gastric irritation and vomiting, it is advisable to hospitalize patients during the loading dose period to enable better monitoring and control of these potential adverse effects. The previously described protocol can also be given rectally, in patients that are unable to take oral medication (56).

A study by Gindiciosi et al. (14) described a loading protocol that consisted of the oral administration 600 mg/kg of KBr split into multiple doses and given over a 48-h period in association with a maintenance dose of 30 mg/kg/day. This protocol was effective in achieving bromide therapeutic concentrations in most patients. Only 5% of the patients in this study vomited during the loading period, without this being impeditive to finish the loading protocol (14).

Longer loading periods with more fractioned dosages might reduce the risk of gastrointestinal signs (44). Patients should be regularly assessed during loading dose protocols for monitoring of side effects that might occur, allowing adjustment or discontinuation of the protocol if necessary.

A "mini" loading dose of 225–250 mg/kg was also suggested to provide rapid adjustments on bromide serum concentrations in patients where this might be required (44).

Intravenous loading can be performed with a NaBr solution and a protocol using a continuous rate infusion (CRI) during a 24-h period to administer a total of 900 mg/kg dose was previously suggested (51). Despite this, clinical studies evaluating the safety and efficacy of intravenous bromide administration in veterinary patients are still lacking.

Intravenous administration of KBr cannot be recommended due to the possible cardiotoxic effects associated with the rapid IV infusion of potassium (57).

5 Efficacy and comparative analysis with other antiseizure medications

Bromide was initially introduced in veterinary medicine as an "add-on" medication for the treatment of epileptic dogs refractory to treatment with phenobarbital and/or primidone, or for cases in which

phenobarbital dosage needed reduction due to liver disease (23, 26, 58–60).

Improvement in seizure control (>50% reduction of seizure frequency) was recorded in 50–83% of the dogs where this medication was added to the antiseizure treatment, providing evidence of its usefulness for this purpose (23, 26).

Reductions in phenobarbital dosage were possible in 35% (23) and 70% (58) of dogs, after the addition of bromide. In a study by Trepanier et al. (27) addition of bromide made possible to discontinue barbiturate treatment (phenobarbital or primidone) in 19% of the dogs.

Royaux et al. (61) described possible benefits of the addition of bromide to epileptic dogs receiving imepitoin. A response rate (decrease of at least 50% in monthly seizure frequency) of 69% (9/13) was reported with the addition of bromide to the treatment of imepitoin-refractory epileptic dogs (61). In 23% (3/13) of the patients, seizure eradication was possible to achieve (61).

The use of bromide in monotherapy, as a first-line antiseizure treatment in alternative to phenobarbital, was also previously described (28). Currently there is only one study evaluating bromide's efficacy in monotherapy for the treatment of epilepsy in dogs and comparing it with phenobarbital (16). First line treatment with potassium bromide was considered to be an acceptable choice, leading to the eradication of seizures in 52% of the dogs (16). Despite this, when compared to bromide, phenobarbital revealed a higher efficacy, leading to the eradication of seizures in 85% of dogs. If the patients continued to experience seizures, these were more likely to decrease in duration for dogs treated with phenobarbital compared to those treated with bromide. In addition to this, and although not statistically significant ($p = 0.059$), the percentage of dogs in which seizures were successfully controlled (>50% reduction in seizure frequency, decreased severity and without unacceptable adverse effects) was also higher for those treated with phenobarbital (15/20; 75%) than for those treated with bromide (15/23; 65%). Improvement of initially witnessed side effects was reported with both medications, but phenobarbital still appeared to be better tolerated, with 20% of the patients receiving bromide continuing to experience vomiting by the end of the study (16).

According to Podell et al. (11), bromide receives a moderate recommendation for its' use in monotherapy and this medication is "most likely" expected to provide an effective treatment.

Compared to canine patients, the efficacy of bromide seems to be less in cats (41). Boothe et al. (41) reported that the use of bromide as monotherapy (4) or in association with phenobarbital (3) lead to the eradication of seizures in 7 of 15 treated cats. However, cats may develop an eosinophilic bronchitis which makes bromide use in cats not advisable, what also limits the current available studies in this species (62).

Studies assessing the efficacy of bromide as an antiseizure medication in horses are currently lacking.

6 Adverse effects and tolerability

Neurological and behavioral signs were the most reported adverse effects among different veterinary species (63).

Sedation, ataxia and/or paresis can occur in patients treated with bromide, particularly with increased serum concentrations (14, 23). Controversially, irritability and restlessness were also reported with potassium bromide treatment in dogs (64).

Non-neurological adverse effects include mainly polyuria, polydipsia and polyphagia, all frequently seen in patients receiving phenobarbital (14, 23).

6.1 Polyphagia

Polyphagia might be related to an increased caloric need or behavioral effect associated with this medication (63). Podell and Fenner (23) noted polyphagia in 7/23 dogs treated with a combination of phenobarbital and bromide once the last one was added to the antiseizure plan. In these patients it was not possible to conclude if polyphagia was due to bromide or the combination of the two medications (23). Polyphagia was also reported by about 20% of the owners of dogs treated with bromide, with this number increasing to around 80% if this medication was combined with phenobarbital (64).

Although weight variations in patients treated with bromide are currently not clear, eating habits and body weight should be closely monitored (63). Polyphagia can be severe to lead to garbage and foreign body ingestion, leading to secondary complications (44, 63).

6.2 Polyuria and polydipsia

Evidence regarding the frequency of polyuria and polydipsia (Pu/Pd) in dogs treated with bromide is controversial. Podell and Fenner (23) reported Pu/Pd in 13 out of 23 dogs receiving potassium bromide in combination with phenobarbital (23). On the other hand, in a study by Chang et al. (64) although Pu/Pd was not noted by the owners of dogs receiving bromide in monotherapy, these adverse effects were more frequently reported in patients receiving multitherapy with phenobarbital and bromide, rather than phenobarbital alone, suggesting that bromide can have a potential for these adverse effects (64). On the other hand, Pu/Pd is a commonly reported adverse effect in the clinical experience of one of the authors (GBC).

6.3 Gastrointestinal signs

Vomiting and diarrhea (including bloody feces) were also described in dogs treated with potassium bromide or sodium bromide but are usually not severe and seldomly indicate discontinuation of treatment (63). Based on anecdotal reports regarding the gastric irritant effect of bromide salts (28), administration in divided smaller doses or in association with food might help preventing vomiting (44, 63).

Bromide was previously associated with an increased risk for the development of pancreatitis (23, 26, 65). Dogs receiving treatment with bromide seem to have a higher risk of having elevated serum canine pancreatic lipase (66) and Gaskill and Cribb (65) reported an increase incidence of pancreatitis from 0.3% to at least 10%, after adding potassium bromide to the treatment of epileptic dogs treated with phenobarbital.

Phenobarbital alone or in combination with bromide can also lead to the development of hypertriglyceridemia in dogs, a known risk factor for pancreatitis (67). Despite this, the impact of

bromide in contributing to hypertriglyceridemia is currently unknown and evidence to support a clear causal relationship between bromide treatment and pancreatitis also seems to still be lacking.

Megaoesophagus has been anecdotally reported (68), although whether there is a causal relation with bromide treatment remains unclear.

6.4 Dermatologic signs

Dermatologic adverse effects are only rarely reported in canine patients and do not appear to be a significant problem in patients receiving bromide therapy (63). Cutaneous lesions included nonsuppurative white macules with scales and pustular dermatitis (63). Panniculitis was reported in two dogs and resolved after discontinuation of bromide treatment (69).

Pruritus not associated with skin lesions was also previously reported (44, 64).

6.5 Bromide toxicosis (bromism)

Bromide toxicosis (bromism) appears to be dose-dependent and linked to high serum bromide concentrations (63, 68).

Rossmeisl and Inzana (68) found a mean bromide serum concentration of 3.7 mg/dL in dogs with clinical signs of bromism, compared to 1.7 mg/dL in control dogs.

Despite this, bromide tolerance seems to vary among individuals and, as a result, cases of toxicosis were also reported with low serum concentrations (40). For this reason, clinical signs should always be used in association with serum bromide concentrations to appropriately judge the tolerability of bromide treatment in a specific patient (63).

Serum bromide concentrations should be regularly assessed to identify possible changes in concentration trends and allow intervention before the development of signs of bromism or breakthrough seizures (68).

Throughout different species, severe bromide toxicity results in manifestations such as depression, alterations in behavior, ataxia, hind limbs paresis, mydriasis, stupor, and coma (63).

In cases where bromide is given in association with phenobarbital, a decrease of 10–30% of the dosage of phenobarbital can reduce the severity of neurological adverse effects in a few days.

Bromide dose reduction, intravenous fluid therapy and induction of diuresis with saline solution (0.9% NaCl) can lead to a quicker improvement in a matter of hours (63, 68).

Breakthrough seizures might occur during the treatment of bromism, which might increase the patient's hospitalization time. Care should be taken while administering intravenous fluid therapy and this should be performed in association with serial monitoring of serum bromide concentrations (68).

6.6 Other

Experimental studies in rats revealed that bromide can disturb the thyroid, testes and adrenal's function (70, 71). Despite this, in the studies by Kantrowitz et al. (37) and Paull et al. (38) bromide revealed no influence in canine thyroid function or morphology.

Bromide should not be used in pregnant or lactating animals since its safety is yet to be assessed in these patients.

6.7 Non-canine patients

In feline patients, cough, is the most frequent adverse effect (53) this is associated with the development of an eosinophilic bronchitis that can be life-threatening (62).

In a retrospective study, Boothe et al. (41) reported adverse effects in 8 out of 17 feline patients receiving bromide. Cough was particularly common (6/17) and lead to the death of one patient, with an overall mortality rate of 5/17. In another study cough was described in 11/26 cats treated with bromide (72). Two of these patients died as a result for severe respiratory signs (72). Onset of cough was reported between 2 weeks to 23 months after starting treatment (41), but was seen to developed as late as 8 years after initiation of treatment (62).

Resolution of cough occurs only after discontinuation of treatment, supporting a relationship with bromide treatment (41, 62). However, time for resolution was reported to be very variable (1–16 months) (41). Corticosteroid treatment might be required to cease respiratory signs in some patients (41, 62) and some might require long-term corticosteroid medication even after cessation of bromide treatment, suggesting that bromide might lead to severe and irreversible chronic inflammatory lower airway disease in some feline patients (62). Nodular pulmonary lesions and endogenous lipid pneumonia with secondary pneumothorax were also previously described (62).

Respiratory adverse effects were rarely reported in clinical studies in canine patients (51, 63).

Dermatitis (bromoderma; idiosyncratic or dose-independent) and vomiting, weight gain and polydipsia (dose-dependent) were the most uncommon adverse effects reported in cats (53).

In horses, bromide has also a sedative and calming effect that can lead to its misuse in competition animals (73). In cattle bromide seems to help decreasing aggressive behaviors (74).

7 Serum level monitoring

Bromide serum concentration monitoring is indicated in patients receiving treatment with this medication. Adequate monitoring allows individualization and optimization of treatment due to the narrow therapeutic ranges and known pharmacokinetic variability between individuals (52).

Serum level monitoring also allows the clinician to determine if medication failure is related to metabolic tolerance (in patients requiring dose adjustment) or functional tolerance (in patients requiring a change of medications). It helps monitoring owner's compliance and prevent possible toxic effects (75).

Bromide serum concentrations should be measured once a steady-state concentration is reached between 6 and 12 weeks after the beginning of treatment with a maintenance dosage (11, 51, 52). In patients showing good seizure control, bromide serum concentrations should be assessed on an annual basis. This assessment should

be anticipated if seizure frequency increases with more than 3 seizures before the next scheduled assessment or if toxicity is suspected (11).

The end result of a loading dose can be assessed 1 week after the administration of a loading dose protocol. Subsequently, another assessment should be performed one-month post-loading to determine the appropriate ongoing maintenance dose (51, 52). The patient's maintenance dose should be increased if there is a decrease of more than 10% in bromide serum concentration between these two measurements (76).

Due to bromide's long elimination half-life, timing of blood sample collection after oral administration is not critical, although samples collected more than 2 h after dosing should help avoiding peak effect variability (35).

The current recommended therapeutic bromide serum concentrations are based in only a few previous studies on the use of this medication (23, 26, 27).

If used as an add-on treatment, in association with phenobarbital or primidone, bromide serum concentrations between 700 and 2,000 mg/L (26) and 880–2,470 mg/kg (23) proved to be effective in improving seizure control.

Similarly, Trepanier et al. (27) found serum concentrations between 810 and 2,400 mg/L to be adequate when bromide was used in association with phenobarbital, and 880–3,000 mg/L when bromide was used in monotherapy.

It is important to mention that the suggested bromide therapeutic serum concentrations should not be seen as an absolute truth. Prospective dose titrating studies are still currently lacking, and it is possible for adequate seizure control to occur with concentrations below those of the expected therapeutic range. Equally (as previously discussed), concentrations above this range do not necessary result in severe side effects or bromism in all patients (16).

To date, determining an accurate serum bromide concentration remains challenging, typically requiring samples to be sent to an external laboratory, which can potentially delay therapeutic decisions (77).

Most analytical methods that are currently used to assess chloride concentrations measure the total halide ion concentration, what includes chloride and bromide. This leads to spurious hyperchloremia (or pseudohyperchloremia) to be frequently recorded when assessing the serum, whole blood or plasma of patients receiving bromide treatment (77, 78).

A study by Woody et al. (79) showed that, in human patients, the degree of pseudohyperchloremia could be used as an indirect method to assess bromide serum concentration in a quicker manner (79). Inspired by these results, Rossmeisl et al. (77) studied the existence of a possible similar relationship between the magnitude of pseudohyperchloremia and bromide serum concentration in bromide treated epileptic dogs. Rossmeisl et al. (77) concluded that in dogs this relationship was unsatisfactory for routine use in practice and bromide dose adjustments should be based on the direct assessment of bromide serum concentration. Despite this, since chloride can easily be measured in-house in most practices, the variation and degree of hyperchloremia can still be used as a general guide to adjust bromide dosage in emergency situations (77).

Bromide concentration can be determined by a spectrophotometric, gold chloride method (80). After precipitation of the proteins in the sample, tri-gold chloride is added to the serum leading to a color change that is related to the level of bromide present in the blood. The resultant color change is then read spectrophotometrically (81). This method carries a risk of falsely record elevated bromide levels due to the presence of other iodides (78). Additionally, this method is laborious and nowadays most laboratories prefer the use of mass spectrometry.

Due to the impact of hemolysis in spectrophotometrical techniques (82), care to avoid erythrocyte damage should be taken when collecting blood samples for bromide measurement.

A study by Mandigers (83) assessed 51 dogs receiving bromide treatment in two different laboratories and revealed a difference between −1111 mg/L to 3,910 mg/L, with a mean difference of −128 ± 728 mg/L between the two laboratories. These results concerningly show that bromide measurements can differ very significantly between different laboratories, even when the same laboratory method is used, providing further evidence on the difficulty in obtaining reliable bromide measurement results.

Mass spectrometry is used to identify and quantify different types of molecules based on their mass-to-charge ratios. In simple terms, mass spectrometers measure the mass of molecules that were previously converted into ions, allowing their precise quantification (84). Once limited to research and specialized clinical laboratories, mass spectrometry is now more readily available and routinely used (85).

8 Drug interactions

Bromide has no known interactions with other common antiseizure medications (81).

Muñana et al. (86) assessed the possible influence of a combination of phenobarbital and/or bromide in the clearance of levetiracetam of epileptic dogs receiving multidrug treatment. The combination of bromide with levetiracetam did not result in any significant recorded pharmacokinetic interaction. This contrasted with the combination of phenobarbital (alone or in association with bromide) with levetiracetam, which led to an increased clearance and lower levetiracetam plasma concentrations (86).

The absence of known interactions between bromide and other antiseizure medications may be attributed to its lack of metabolism within the patient's body (81).

Bromide exhibits its most significant and well-known interaction with chloride, which in turn is influenced by the patient's diet. Diets with higher chloride content may result in faster elimination of bromide (87).

Trepanier and Babish (87) suggested that the predicted mean daily doses of bromide needed to maintain an adequate serum concentration were significantly higher for patients with higher chloride content in their diet (87). For this reason, there is a general recommendation for the ingestion of chloride to be maintained constant in human and veterinary patients receiving treatment with bromide (87, 88). Dogs with adequate seizure control can experience recurrence of seizures after a sudden increase in chloride intake through a diet change and consequent drop in serum bromide concentrations (89).

A study by Lichtenauer et al. (90) evaluated a possible relationship between the proximity of a dog's residential area to the coast and the dose of potassium bromide required to maintain adequate serum bromide concentrations (90). It is thought that dogs living close to the sea can be exposed to air with higher concentrations of salt in the form

of aerosols (91, 92). Despite the lack of statistically significant results, this study identified a trend where dogs living by the sea appeared to require higher doses of potassium bromide, suggesting that the impact of chloride in dogs treated with bromide might go beyond nutrition (90).

In cases of bromide toxicity, this known interaction can be used to increase the excretion of bromide by the administration of high amounts of sodium or ammonium chloride (43), as previously described. Furosemide was also formerly used to promote bromide diuresis in cases of bromism and bromoderma (49, 50). Its exact impact on bromide clearance is still unclear (76).

Since bromide is eliminated by the kidneys, renal disease can lead to an inappropriate rise in bromide serum concentration and consequent bromide toxicity (50). Regular monitoring of the kidney function is therefore recommended in patients receiving bromide treatment (50).

9 Discussion

Managing epilepsy effectively in veterinary patients remains a challenging aspect of veterinary neurology since poorly controlled seizures can severely impact both the patient's and the owner's quality of life. Additionally, a 20–30% of epileptic dogs are refractory to treatment (23, 27, 93, 94), a phenomenon also observed in human medicine (95).

It's no surprise that the field of epilepsy continues to receive extensive research efforts in order to find additional therapeutic options that can potentially enhance the management of this condition.

Since its introduction by Schwartz-Porsche and Jürgens (26) that bromide continued to have an important role in the treatment of epileptic dogs, refractory to phenobarbital therapy.

Bromide's long half-life can be seen as an advantage since the administration of loading doses can still allow the clinician to provide the patient with therapeutic serum concentrations in a timely manner (14) and maintenance doses can be performed only once daily, possibly contributing to owner's compliance.

Bromide follows phenobarbital and imepitoin (for idiopathic epilepsy) as the ASM with "moderate recommendation and most likely to be effective treatment" when used in monotherapy (11).

Use in monotherapy can be particularly interesting in patients that have a contraindication for the treatment with first line ASM (e.g., phenobarbital in case of hepatic disease; structural epilepsy in case of imepitoin). Despite this, it is important to note that evidence to support the use of bromide in monotherapy is limited to one single study by Boothe et al. (76).

Similarly, the currently accepted therapeutic ranges as an "add-on" treatment or in monotherapy are only based on a few studies where these serum concentrations appeared to be effective (16, 23, 26, 27). Diets were also not uniform throughout the patients of these studies, possibly impacting bromide half-life and consequently the level of maintenance dose required.

Future prospective studies assessing bromide efficacy as monotherapy in epileptic dogs receiving a stable and uniform diet could help to better clarify its possible role as a possible first-line ASM and deepen our understanding of recommended serum therapeutic ranges.

Nonetheless, the noticeable variable sensitivity to bromide between different canine patients (40, 63), highlights the need for a tailored and individualized treatment plan, with the currently known therapeutic ranges and suggested doses to be used as an initial guide.

Due to the challenges presented in assessing serum bromide concentrations, clinicians should aim to work with a trusted laboratory and avoid comparing results obtained from different laboratories. Furthermore, as the Tri-gold measurement method carries a risk in getting incorrect values, it is advised to use laboratories that use mass spectrometry as a measurement method (96).

Owners and clinicians alike should be aware of the impact abrupt changes in chloride intake can have in seizure control. This is important to remember when these patients receive treatment for other conditions, particularly when intravenous fluid therapy is used. Bromide serum concentration can drop in a matter of hours possibly leading to breakthrough seizures (68). Bromide concentration should be closely monitored, and dose adjustment might be required during these periods.

Adverse effects seem to be the main disadvantage associated with bromide treatment, but these appear to be better controlled and tolerable in dogs when compared to people.

Although some authors advise that gastric irritation caused by bromide and consequent vomiting can be limited or controlled by dividing daily dosage or with its administration in association with food (28, 44, 63), to the authors' knowledge, prospective studies assessing the effect of these measures on the frequency of gastrointestinal side effects are still lacking.

In human patients, neither phenobarbital nor bromide were associated with the development of pancreatitis (97). Studies assessing the risk of pancreatitis in bromide treated dogs are limited (65–67) and although this still appears to be a possible complication of the use of this medication, evidence for a causal effect has not yet been obtained. It is currently unclear whether the possible risk of pancreatitis might be due to onset of pica and polyphagia, leading to alimentary indiscretion and associated complications (63). In any case, it seems wise to monitor for signs of pancreatitis in canine patients treated with bromide. The value of monitoring the patient's triglyceridemia in preventing or anticipating signs of pancreatitis is also unknown and could reveal an interesting field for future studies.

Due to the known possible weight changes, body weight should also be closely monitored.

In feline patients, use of bromide cannot be advised due to the severity of adverse effects. Studies assessing bromide efficacy are also (and should continue to be) limited due to this (41, 62).

To the authors knowledge, studies assessing bromide use and efficacy in horses are not available and the possible role of bromide as a treatment of epileptic seizures in this species is still unknown.

Treating epilepsy requires tailor-made medicine. Bromide remains a cornerstone in the treatment of canine epilepsy since its introduction in the 1990s. Despite its widespread use, studies supporting the currently recommended serum therapeutic concentrations and its efficacy when used as monotherapy are still scarce. Future prospective research on bromide use, monitoring, interactions and side effects could enhance our understanding of this medication even further and ultimately help empowering clinicians and owners with greater confidence and efficacy in the management of canine epilepsy. Nonetheless, we can identify some potential good, bad and ugly aspects of the use of this medication. The good is that

bromide has proved efficacy as an add-on treatment in cases of canine refractory idiopathic epilepsy and might also serve as an alternative first-line ASM in selected cases. The currently known challenges in measuring and monitoring bromide serum concentrations, make it difficult to tailor dosages to the individual patient and represent one of the downsides (or bad aspects) of its use. Finally, the ugly side of bromide use seems mainly associated with cases where clinicians failed to adequately adjust the treatment to the patients' needs leading to a wide range of adverse effects. These might impact the quality of life of both dogs and owners to an unacceptable degree. The severity of adverse effects can be such that bromide was largely abandoned in human medicine and is currently contraindicated in cats. Continuous and accurate monitoring of bromide serum concentrations is necessary to maximize its therapeutic properties and ensure its safe use.

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Magnetic resonance imaging in canine idiopathic epilepsy: a mini-review

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Magnetic resonance imaging (MRI) in an integral part of the diagnostic workup in canines with idiopathic epilepsy (IE). While highly sensitive and specific in identifying structural lesions, conventional MRI is unable to detect changes at the microscopic level. Utilizing more advanced neuroimaging techniques may provide further information on changes at the neuronal level in the brain of canines with IE, thus providing crucial information on the pathogenesis of canine epilepsy. Additionally, earlier detection of these changes may aid clinicians in the development of improved and targeted therapies. Advances in MRI techniques are being developed which can assess metabolic, cellular, architectural, and functional alterations; as well alterations in neuronal tissue mechanical properties, some of which are currently being applied in research on canine IE. This mini-review focuses on novel MRI techniques being utilized to better understand canine epilepsy, which include magnetic resonance spectroscopy, diffusion-weighted imaging, diffusion tensor imaging, perfusion-weighted imaging, voxel based morphometry, and functional MRI; as well as techniques applied in human medicine and their potential use in veterinary species.

KEYWORDS

MRI, epilepsy, dog, neuroimaging, brain, seizure

Introduction

Epilepsy is one of the most common chronic neurologic conditions in dogs with an estimated prevalence of 0.62–0.75% in the general dog population (1–3). The diagnosis of idiopathic epilepsy (IE) is based on the exclusion of other underlying etiologies for which magnetic resonance imaging (MRI) is essential to the diagnostic workup and considered part of the Tier II confidence level for the diagnosis of IE as put forth by the International Veterinary Epilepsy Task Force (4). Three main aims of advanced MRI in the epileptic animal are: (1) to rule out causes of epileptic seizures which may be treatable with means other than antiseizure medications (ASM) only (e.g., inflammatory or infectious brain disease), (2) to identify lesions caused by epileptic seizures which are not themselves the source of seizures, and (3) to provide data to further advance the field of research into the pathogenesis and/or treatment of epilepsy (5).

However, conventional, or structural MRI lacks the specificity to identify many disease processes due to significant overlap in imaging characteristics and lesion morphology between intracranial etiologies in dogs (6, 7). As such, the study of idiopathic (or "non-lesional" in human medicine) epilepsy with MRI is challenging as this condition often presents with a normal-appearing brain (8). Structural abnormalities are identified in only 2.2% of dogs less than 6 years of age with epileptic seizures (9). There are reports of visible, or invisible, but statistically

identifiable findings in canine epilepsy. Visible post-seizure changes have been documented in both idiopathic and structural epilepsies and can include regions of T2W or fluid attenuated inversion recovery (FLAIR) hyperintensity and T1W iso-to hypointensity with variable contrast enhancement, with the majority of these changes being bilateral and symmetric (9–14). Structures specifically involved include the hippocampus, cingulate gyrus, and piriform lobe (10–12). These changes are often transient, and likely represent a combination of cytotoxic and vasogenic edema associated with increased energy metabolism, hyperperfusion, and cell swelling as a consequence of the ictal activity, but they do not represent the area of the cortex from which seizures arise and propagate, called the epileptogenic zone (8, 10–12).

The concept of the epileptogenic zone was initially proposed in people by Lüders et al. (15), and has since been proposed in canine epilepsy to be defined as "the region of cortex that can generate epileptic seizures and removal or disconnection of which should lead to seizure freedom," and cannot be identified on routine anatomical imaging (8, 16). The epileptogenic zone consists of five different abnormal cortical zones: the symptomatogenic zone, the irritative zone, the seizure-onset zone, the structural abnormal zone (epileptogenic lesion), and the functional deficit zone (8). The functional deficit zone is defined as "the area of the cortex that is functionally abnormal in the interictal period." This zone not only relates to structural (MRI visible) lesions but also to microstructural lesions and true areas of functional abnormalities, as occur in IE (8). Therefore, imaging modalities able to detect the epileptogenic zone are both essential for accurate presurgical evaluations and helpful in understanding the pathophysiology of canine and feline epilepsy.

In addition to identifying non-structural changes that may aid in the identification of the epileptogenic zone, there is growing evidence of structural changes within the hippocampus, temporal lobes, or white-to-gray matter ratios in dogs with IE which do not correlate with epileptogenic zone (17). As such, the International Veterinary Epilepsy Task Force published a consensus statement with recommended MRI protocols in the diagnosis of canine IE which may allow the detection of subtle lesions not apparent with existing sequences, imaging planes, or particular techniques. The developed protocol aims to facilitate improved evaluation of areas susceptible to the generation and perpetuation of seizures (5).

Advances in MRI techniques may also aid more detailed examination of these areas, including the ability to assess metabolic, cellular, architectural, and functional alterations, as well as alterations in tissue mechanical properties (18–20). This mini-review focuses on novel MRI techniques being utilized to better understand canine epilepsy, which include magnetic resonance spectroscopy (MRS), diffusion-weighted imaging (DWI), diffusion tensor imaging (DTI), perfusion-weighted imaging (PWI), voxel-based morphometry (VBM), and functional MRI (fMRI); as well as techniques applied in human medicine and their potential use in veterinary species.

Techniques

Diffusion weighted imaging and diffusion tensor imaging

Diffusion-based MRI, such as diffusion-weighted imaging (DWI) and diffusion tensor imaging (DTI), can identify variation in diffusion characteristics of intra-and extracellular water compartments, and exchange of water across permeable boundaries, based on the sensitization of the Brownian motion of water molecules. In the brain, the movement of water molecules in three compartments contributes to the measured degree of diffusion: intravascular perfusion, extracellular diffusion, and intracellular diffusion (21, 22). When this movement of water molecules is obstructed, it becomes anisotropic, or directionally dependent (23, 24). Assessing this anisotropy allows a significant amount of data indexes to be generated, including fractional anisotropy (FA) and apparent diffusion coefficient (ADC) (24, 25). Fractional anisotropy represents the amount of diffusional asymmetry in a voxel, and is a marker of white matter integrity (22, 26). Thus, it is a highly sensitive measure of the microstructural integrity of fibers. ADC represents the average magnitude of molecule displacement at any diffusion direction determined, and can be utilized as a marker of pathologic tissue changes (22) (Figures 1A–C).

Epileptic seizures have been shown to induce cytotoxic edema through excitotoxicity and thus, DWI is highly sensitive to neuronal damage secondary to seizure activity. Therefore, this technique is being applied in human and veterinary medicine to provide additional information regarding ongoing pathologic changes secondary to seizure activity (8, 22). A reduction in diffusion in human patients with temporal lobe epilepsy (TLE) has been described during the ictus and postictally, whereas increased diffusion has been observed in cases of suspected hippocampal sclerosis (21, 27, 28).

Studies in veterinary medicine have employed diffusion MRI techniques to aid in the identification of the epileptic zone or to identify areas of potential brain damage secondary to epileptic seizure activity (8, 21, 29). A study by Hartmann et al. assessed the feasibility of interictal DWI in dogs with IE to assess the distribution of diffusion in comparison to healthy dogs. Significantly increased diffusion was found in the piriform lobe (including amygdala) of epileptic dogs, proposed to be secondary to the loss of structural organization and expansion of extracellular spaces (21). These findings further support that there are changes at the cellular level in the brains of epileptic dogs. While DWI may be a promising technique in the evaluation of epileptic dogs lacking gross MRI abnormalities, these changes are likely transient. Experimental data in humans shows that ADC changes on MRI may last for a few days with the diffusion normalizing in most cases (27, 30, 31). Indeed, in an experiment of kainic acid-induced complex partial status epilepticus in dogs, a reduction in ADC was noted 3 and 6 h post-induction with increased diffusion after 12 and 24 h and normalization 48 h later (29). Therefore, the addition of imaging techniques that may detect more long-standing cellular changes is warranted as part of the diagnostic imaging assessment in dogs with epilepsy.

Similar to DWI, diffusion tensor imaging (DTI), enables the quantification of diffusion of water, but also allows the characterization of the degree and direction of anisotropy (32, 33). Following the collection of this data, tensor maps are generated and tractography can be performed. DTI is a method where connectivity maps between adjacent voxels are linked if the tensors are oriented in the same direction (23, 24, 34). Colors are then assigned to nerve fiber tracts depending on the direction of water displacement and this fiber tracking allows depiction of white matter tracts; comparison between normal and diseased fiber tracts enables quantification of white matter changes due to damage (35). Typically, the colors represent the predominant orientation of the fibers in a three-dimensional

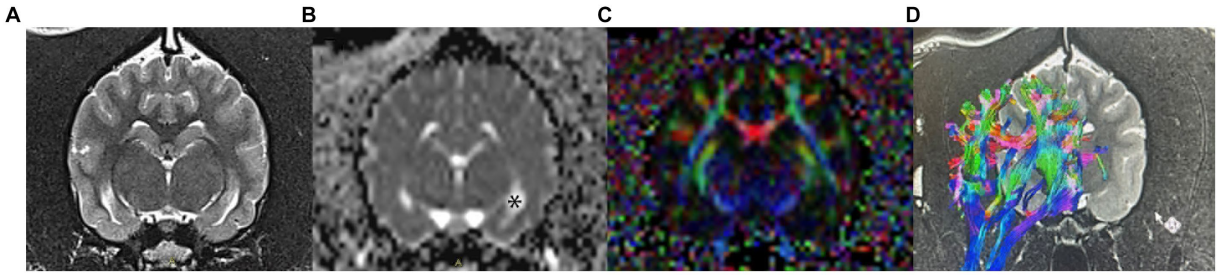

FIGURE 1
Examples of DWI and DTI images obtained in a healthy dog. **(A)** conventional T2-weighted image at the level of the hippocampus; **(B)** apparent diffusion coefficient (ADC) map; areas of unrestricted water diffusion, such as CSF in the lateral ventricles (*), have a high signal intensity on ADC maps. Additionally, gray matter has a higher ADC than white matter **(C)** fractional anisotropy (FA) color map measuring changes in water diffusion within the white matter tracts; **(D)** tensor tracking algorithm of the fibers of the corona radiata. Red indicates right–left direction, green indicates dorsoventral, and blue indicates a rostro-caudal direction.

coordinate system along the axes of space (x, y, and z); red indicates right–left direction, green indicates dorsoventral, and blue indicates a rostro-caudal direction (Figure 1D). DTI has been applied in human medicine in the assessment of TLE, and has shown asymmetry in anisotropy as well as abnormalities not restricted to the temporal lobe (33, 36). In veterinary medicine, DTI has been utilized to establish the white matter tracts in the canine (and feline) brain (37, 38). However, there is only one study on the application of DTI in canine epilepsy. Beckmann et al. performed DTI in dogs affected by IE suffering from generalized tonic–clonic seizures to determine whether white matter diffusion is altered in these patients. The findings of this study showed subtle changes in DTI between dogs with IE and healthy dogs, particularly in the cingulate gyrus. In people with TLE, those suffering from generalized epilepsy syndrome have less pronounced changes than those with TLE. Therefore, these changes may also be less pronounced in dogs with generalized tonic–clonic seizures. The pathophysiology of these white matter changes is unknown, and may represent either a change occurring before the development of epileptic seizures, or a secondary effect (26).

Magnetic resonance spectroscopy

Magnetic resonance spectroscopy (MRS) is a noninvasive technique that provides quantitative measurement of specific metabolites in the brain, and may detect alterations before structural changes are observed (17, 39–41). These metabolites can be identified at 1.5 and 3 Tesla (T) MRI and include: include N-acetyl aspartate (NAA), choline (Cho), creatine (Cr), myoinositol (mI), and glutamate (Glu) and gamma-amino-butyrate (GABA) (40, 41) (Figures 2A,B). The dominant peak in the normal human brain spectra is NAA, which is restricted within neurons, axons, and myelin (42, 43). Therefore, it is considered a neuronal marker for brain neuronal health, viability, and number of neurons (17, 39, 42, 43). Choline (cho) is involved in the synthesis and decomposition of cell membranes and is therefore a marker for membrane damage and gliosis (23, 43). Creatine (Cr) plays a role in energy metabolism and while it is found in all types of neuronal cells, it is in greatest concentration within glial cells (43). Myoinositol (mI) is a glial marker and increased levels indicate glial changes and osmolarity disturbances (43). Glutamate and glutamine peaks overlap and are often measured together as glutamate-glutamine complex (Glx) (17, 44, 45). Increased levels of Glx have neurotoxic potential (23, 39). Metabolites that can be identified at 3 T and are of specific interest in epilepsy are glutamate, GABA, and NAA (39, 46).

In veterinary medicine, MRS has been used to determine the effects of ketamine on brain, specifically thalamic, metabolites in dogs with IE (44) and in the assessment of post-ictal metabolite changes in a generalized seizure model (47). Currently, there are two prospective studies which utilized MRS to evaluate metabolic changes in dogs with IE (17, 39). Olszewska et al. evaluated the interictal metabolic activity of the temporal lobe in dogs with IE compared to healthy controls at 1.5 T. NAA-to-choline (NAA/Cho), choline-to-creatine (Cho/Cr), and choline-to-NAA (Cho/NAA) ratios were determined in both hemispheres and compared to the control population. No significant differences in metabolite ratios were detected between epileptic and control dogs. However, higher values of NAA/Cho were noted in closer proximity to the last seizure whereas the Cho/NAA values were lower in dogs with recent seizures, suggesting that these changes may be temporary (17).

A recent prospective, case–control study assessed and compared MRS spectra within the thalamus of healthy dogs and dogs with IE, focusing on NAA and Glx as well as comparing differences in the spectral data of IE dogs with and without ASMs (39). This study found reduced NAA/Cr in IE dogs on ASMs compared to both healthy controls and IE dogs not on ASMs, as well as reduced Glx/Cr values in IE dogs on ASMs therapy compared to dogs without. In humans with generalized tonic–clonic epileptic seizures, reductions in NAA were more severe in those experiencing more seizures and therefore, in this population of dogs with IE, this finding may reflect what is observed in people (39, 48, 49).

Magnetic resonance elastography

Magnetic resonance elastography (MRE) is a rapidly developing imaging technique used to quantitatively assess the mechanical properties of tissues *in vivo*. As such, it is considered an image-based counterpart to palpation, commonly used by both physicians and veterinarians to diagnose and characterize diseases. MRE obtains information about tissue stiffness by using a special MRI technique involving three steps: (1) generating shear waves in the tissue, (2) acquiring phase-sensitive MR images to detect the propagating waves,

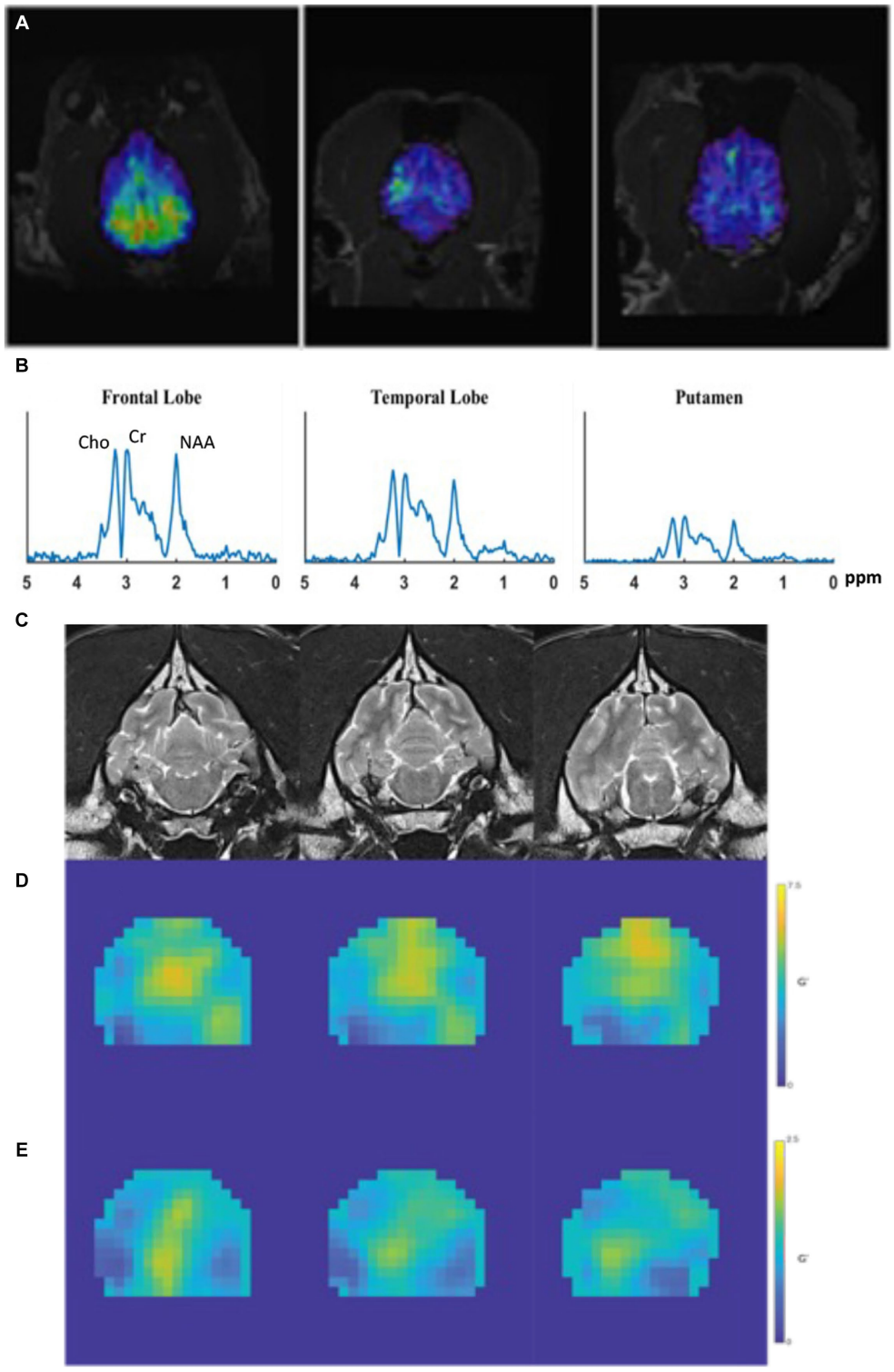

FIGURE 2
Examples of MRS and MRE performed in a healthy canine brain. **(A)** Dorsal MPRAGE of metabolite maps (NAA, overlaid on the corresponding anatomical MRI images) from a control dog demonstrating volumetric metabolite mapping. **(B)** Regional spectra from a dog showing peaks of Cho, Cr, and NAA from various brain regions. **(C)** T1-weighted coronal images obtained prior to MRE acquisition which are used for mapping of elastograms; **(D,E)** quantitative stiffness maps (elastograms) of the **(D)** shear modulus, and **(E)** loss modulus. In both **(D,E)**, areas of highest stiffness are yellow and areas of low stiffness are blue.

and (3) processing the images to generate quantitative stiffness maps, called elastograms (20, 50) (Figures 2C–E).

Only 1 study has investigated brain viscoelastic changes in people with epilepsy. Huesmann et al. performed MRE in people with mesial

temporal lobe epilepsy (MTLE) compared to healthy volunteers and found the hippocampus ipsilateral to the epileptogenic regions was stiffer than the contralateral part, and the stiffness ratio between hemispheres was higher in patients with MTLE compared to healthy participants. This shows that MRE is not only applicable in epilepsy, but it may be sensitive to microstructural pathology which could be present before grossly observable changes in hippocampal volume, an established imaging biomarker in MTLE (51). To date, there is only a single published abstract on the capability of performing MRE in the canine cadaveric brain, which demonstrated variation in tissue stiffness across regions, with a mean whole brain tissue viscoelasticity ± standard error of 2.99 ± 0.30 kPA. Thus, showing promise for the use of this technique in veterinary neuroimaging (52).

Perfusion weighted imaging

Perfusion-weighted imagined (PWI) allows the assessment of blood volume flow in the brain through the determination of cerebral blood volume (CBV), cerebral blood flow (CBF), mean transient time (MTT), time of arrival (T0) to the region of interest (ROI), and time to peak concentration (TPP) (8, 53, 54), though the use of an exogenous contrast medium tracer injected intravenously, such as a gadolinium chelate (53, 55).

In people with TLE, PWI findings demonstrate hyperperfusion during ictus and hypoperfusion postically (31, 56–61). There are two canine IE studies, in which PWI was assessed interictally with similar findings to what has been documented in people. Hartmann et al. demonstrated that dogs with IE have decreased brain perfusion compared to healthy dogs (54). Nagendran et al. noted various changes in perfusion with some IE dogs demonstrating hyperperfusion and others having hypoperfusion.

Arterial spin labeling

Arterial spin labeling (ASL) allows the quantification of brain perfusion (CBF) non-invasively, without the use of any contrast agent or ionizing radiation. Blood water entering the brain is magnetically labeled as an exogenous tracer (62, 63). The perfusion signal is obtained by subtracting the labeled image from a control image in which blood has not been labeled, resulting in an image with the signal intensity proportional to CBF (i.e., reflecting the amount of blood delivered to each voxel). This technique has been applied in people in the assessment of TLE and frontal lobe epilepsy, and in presurgical planning in MRI-negative focal epileptic patients (62–66).

In animals, brain ASL-MRI has been used widely in experimental research (67–71). Recently, Hoffman et al. successfully performed ASL in a large population of dogs and cats with a success rate of 95% in animals with a normal brain MRI. Future directions would include the application of this technique to potentially characterize various brain diseases in dogs and cats, including epilepsy (72).

Voxel based morphemtry

Voxel-based morphometry (VBM) is an automatically computational quantitative MRI analysis technique used to detect differences in brain morphology across different subjects (73, 74). In people with TLE, VBM has demonstrated a reduced or increased volume of several gray matter structures, typically ipsilateral to the side of seizure onset (73).

There are limited volumetric studies in veterinary medicine, largely based on the significant variation in head and brain shape between breeds (75). Recently, Frank et al. assessed differences in gray matter volume (GVM) between healthy and epileptic Beagles and found a significant reduction in GMV in several areas of the brain in epileptic dogs (74).

Functional MRI

Functional MRI (fMRI) is the representative functional imaging technique in human neuroscience and utilizes magnetic susceptibilities of oxyhemoglobin and deoxyhemoglobin (blood oxygen level-dependent [BOLD] contrast). Areas with increased brain activity have a greater metabolic demand and thus more oxyhemoglobin. fMRI has been performed in humans with TLE and often combined with electroencephalography (EEG) (8, 76, 77). Resting-state fMRI (rs-fMRI) is based on low-frequency fluctuations in the BOLD signal when the brain is at rest (78). In human epilepsy, rs-fMRI has demonstrated altered functional connectivity in large-scale networks, including attentional (79), perceptual (80), and default mode (81–83); with the most widely studied network in epilepsy being the default mode network (DMN) (84).

To date, the only awake fMRI studies in canine patients have been experimental and require extensive training for the patients to remain immobile, leading to the use of a small number of dogs (85–87). There is limited research on the use of rs-fMRI in diagnosing canine IE, but one study found significantly increased functional connectivity in the anterior DMN in dogs with IE compared to healthy controls (88).

Limitations

Many of these modalities have yet to be established in the canine brain, and more work is needed to optimize these sequences for use in veterinary patients. Many of these techniques require technical skills, which significantly limits their integration into clinical practice in both veterinary and human medicine. Automated software for brain extraction and data processing has been developed in human medicine. A limitation in veterinary medicine is the highly variable anatomy of the canine brain (5, 89). Milne et al. assessed three different atlas-based segmentation techniques in the three basic canine brain shapes (brachycephalic, mesaticephalic, and dolichocephalic) (75). This study demonstrated that the use of manual brain extraction techniques with the application of brain shape-specific templates is highly accurate and repeatable. A limitation of this study is that while the MRIs were structurally normal, some of the patients enrolled had neurologic signs or behavioral changes. Therefore the effect of any undetected structural pathology cannot be determined (75). Further work is needed in developing automated brain extraction techniques in the canine brain.

Conclusion

Canine IE is often a diagnosis of exclusion, and in most patients, potential changes associated with epileptic seizure activity may be missed on routine MRI. Therefore, more novel imaging techniques may be required to detect lesions in patients with structurally normal brains visualized with traditional MRI. These novel techniques provide clinicians and researchers opportunities to improve diagnostic capabilities and expand knowledge of targeted therapeutic planning and monitoring in both human and canine epilepsy.

Author contributions

KF: Supervision, Writing – original draft, Writing – review & editing. AB: Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Surgical management of primary and idiopathic internal hydrocephalus in dogs and cats

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Ventriculoperitoneal shunt placement is an effective method to treat internal hydrocephalus in dogs and cats. Although it has a long history in veterinary medicine, the technique continues to evolve. Despite continuing attempts to reduce the incidence of associated complications, shunt failure remains a major problem, and often leads to multiple hospital admissions. This review gives an overview about current knowledge of ventriculoperitoneal shunting techniques in animals, applicable shunt hardware as well as shunt-associated complications and their prevention and treatment.

KEYWORDS

canine, feline, hydrocephalus, magnetic resonance imaging, VPS

Introduction

Congenital internal hydrocephalus is a common malformation in dogs and cats (1–3). However, internal hydrocephalus is heterogeneous in nature and can be due to a wide spectrum of other pathological entities (4). Underlying causes include primary brain malformations (cysts, aqueductal stenosis, interventricular septae etc.), as well as secondary causes, including intraventricular hemorrhage, intracranial tumors, and infectious diseases. In many cases, the underlying cause remains undetermined (idiopathic hydrocephalus) (4). Untreated hydrocephalus can lead to many clinical signs including ataxia, blindness, behavioral abnormalities, and vestibular signs, and can also lead to death (5). Ventriculoperitoneal shunting (VPS) is currently the best treatment option (3, 6). Whereas VPS is one of the most common neurosurgical procedures in humans (7), there is less experience with this technique in hydrocephalic animals. Despite perfect shunt placement and function, short-term and long-term complications may prevent improvement of clinical signs and survival of the animals (8–10). Thorough preoperative planning, selection of appropriate hardware, and postoperative monitoring can help to reduce the occurrence of complications and the need for shunt revision. In this review, we summarize current knowledge on VPS in dogs and cats. The most common complications of VPS as well as strategies for prevention and their treatment are discussed.

Review

Surgical technique and equipment

The purpose of VPS is to divert excess cerebrospinal fluid (CSF) from the cerebral ventricles to the abdominal cavity. Although all available shunt systems have the same basic

components in order to achieve this, they differ in a few details. Our experience is mostly confined to one particular shunt system.

The ventricular catheter is a flexible silicone tube that is placed in the lateral ventricle. It contains a mandrel that helps to avoid kinking of the catheter during implantation through the cerebral cortex. The ventricular catheter contains regular holes arranged in several drainage segments, each containing four holes that take up CSF (Figure 1A). The distance from the tip is marked on the catheter allowing for setting of the appropriate insertion depth. A smaller number of drainage segments in a short distance to the catheter tip (Thomale catheter, Figure 1B) is used in humans in order to avoid potential parenchymal contact with the drainage segments (see below) (11). This ventricular catheter type is especially useful for smaller dogs and cats with smaller ventricular dimensions.

The ventricular catheter has variable types of shunt deflectors, allowing 90° deflection of the ventricular catheter in the burr hole without kinking the catheter (Figures 1C,D). These are adjustable on the catheter and help to pre-set the maximum depth of the ventricular catheter. The shunt systems usually incorporate a reservoir consisting of a solid base and a silicone dome (Figure 2A). Punctuating the dome allows sampling of CSF out of the shunt tube. Some reservoirs include a non-return valve in the proximal inlet connector that avoids flow in the direction of the proximal catheter during the pumping procedure (control reservoir; Figure 2B). Pressing the dome results in a CSF pressure wave towards the valve, which is a common site of obstruction in humans. A reservoir can be a separate part of the shunt, but can also be combined with a valve (Figure 2C).

An important component of the shunt system is a valve that regulates the driving pressure through the shunt. To avoid overshunting (see below), the shunt systems contain valves that act like on-off switches, opening when the intraventricular pressure (IVP) exceeds the valve's opening pressure, allowing egress of CSF until IVP falls below the opening pressure (differential pressure valve system). Available valves either use a ball-in-cone and spring mechanism, in which the resistance of the spring reflects the opening pressure or involves a membrane that closes the outlet of the valve, but gradually deforms in response to increasing pressure (diaphragma valve, codman Medtronic) (12) (Figure 3).

These differential valves are available in several prefixed pressure ratings. Valves with opening pressures between 0–5 cm of H_2O are defined as low-pressure, 5–10 cm of H_2O as medium-pressure, and 10–15 cm of H_2O as high-pressure valves (2). The drainage rate of the shunt system is not only determined by the IVP, but also by the intraabdominal pressure and the hydrostatic pressure column in the catheter (13). In humans overdrainage can occur in the standing position, when the hydrostatic pressure in the vertical catheter causes a siphon effect (14). To avoid overshunting, gravitational valves are used in humans and dogs (10). As soon as the patient (human) moves into an upright position, the gravitational unit inside the valve (antisiphon device) closes and the total opening pressure of the valve is significantly increased (Figure 4).

An alternative strategy to avoid overshunting is the gradual selection of the optimal opening pressure, which is the highest setting that still allows for CSF drainage, and which is reflected by the patient's clinical improvement in human neurosurgery. Such programmable shunt systems allow the opening pressure to be gradually altered non-invasively using a special magnetically activated mechanism. The opening pressure is gradually decreased until the clinical signs of the patient improve (15). Programmable shunt systems and the necessary equipment for external pressure adjustment are extremely costly, which limits their use in companion animals.

Flow-regulating shunt systems can avoid overshunting by limiting the total amount of CSF that is drained from the ventricles per hour. These valves increase the hydrodynamic resistance when CSF flow increases. Some examples include the SiphonGuard device (Codman) and the Orbis-Sigma OSV II valve (Integra Neuroscience) (Figure 5). The great advantage of flow-regulating devices is that they can be placed at any level of the tube and require no particular orientation (horizontal vs. vertical), which makes them useful for quadrupeds. They also avoid non-postural overdrainage caused by physical exertion, coughing, abdominal underpressure, etc. However, the minimal regulated flow volume of 5 mL per hour is adapted to a human CSF production rate and exceeds the canine or feline CSF production rate (13). This technical characteristic rather limits the benefit in dogs and cats.

Indication for surgery

The diagnosis of congenital hydrocephalus is usually straightforward, as ventricular expansion is severe and causes craniofacial dysmorphia and doming of the calvaria in affected animals. In subadult or adult animals in which these skull changes might not be seen, the diagnosis of hydrocephalus may be difficult to establish, particularly in small brachycephalic dog breeds that tend to have relatively larger ventricles (ventriculomegaly) in comparison to mesocephalic dogs (16). It has been widely accepted that this increase in ventricular volume is not associated with clinical signs and VPS is not indicated (17–20). Morphological studies have shown, however, that dogs with ventriculomegaly have reduced cerebral white matter volumes and impaired periventricular perfusion, as in clinically relevant hydrocephalus (18, 21). Thus, ventriculomegaly should be seen as a form of chronic and potentially arrested hydrocephalus that may even show normal intraventricular pressure (18, 21). However, it is currently unclear as to whether ventriculomegaly is a progressive or non-progressive condition, and what the negative consequences of a slowly dilating ventricular system would be. There is no threshold level of ventricular volume that discriminates between internal hydrocephalus and ventriculomegaly, and even high grades of ventricular distension were diagnosed without the presence of clinical signs (16). It is therefore important to guarantee that the presented clinical signs are caused by ventricular distension and not by other brain diseases.

Small brachycephalic dogs are predisposed to meningoencephalitides of unknown origin (MUO) (22, 23), which might be present in addition to ventricular enlargement. MUOs might remain undetected, especially if CT is used as an imaging modality to diagnose internal hydrocephalus. Hence, it is thought that ventriculomegaly is sometimes misdiagnosed as relevant internal hydrocephalus and interpreted to be the cause of clinical signs in dogs that are affected by inflammatory/

Abbreviations: CSF, Cerebrospinal fluid; CT, Computed tomography; LPS, Lumboperitoneal shunt; MRI, Magnetic resonance imaging; IVP, Intraventricular pressure; VPS, Ventriculoperitoneal shunt.

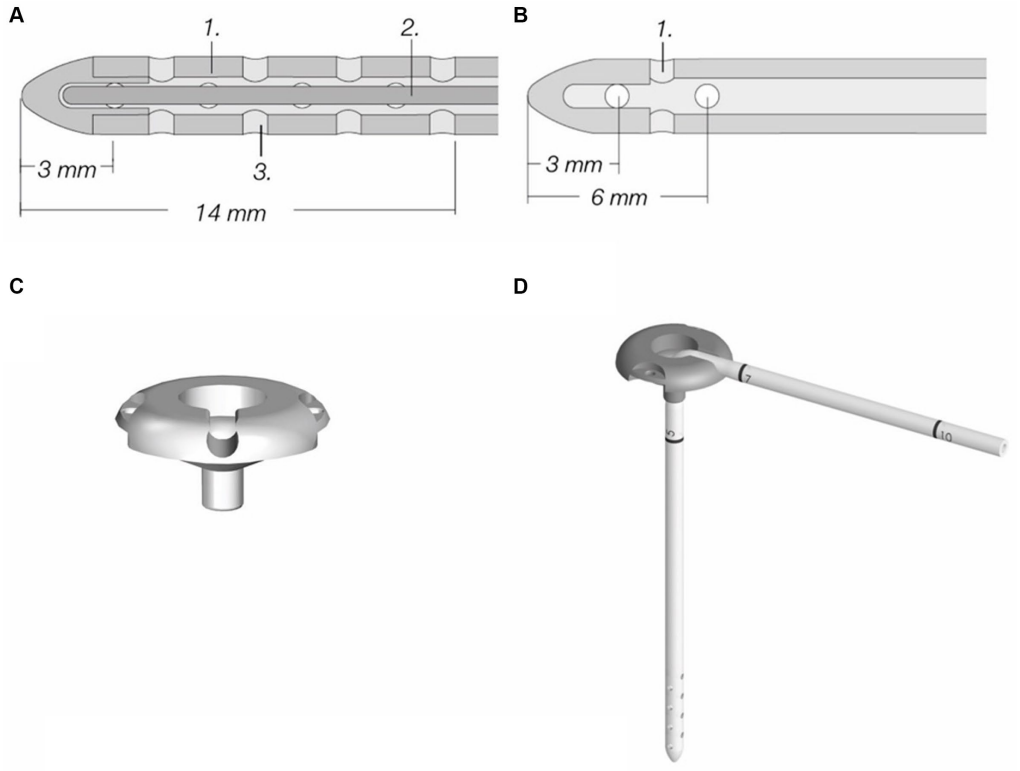

FIGURE 1
Components of a ventriculoperitoneal shunt system (MIETHKE GmbH & Co. KG, Potsdam, Germany). **(A)** Standard ventricular catheter with mandrel, containing eight drainage segments; inner diameter is 1.2 mm, outer diameter is 2.5 mm. **(B)** Thomale catheter with only three drainage segments, inner and outer diameters are the same as in standard catheters. **(C)** Shunt deflector. **(D)** Shunt deflector with ventricular catheter. Permission granted by Christoph Miethke GmbH & Co. KG, Potsdam, Germany; www.miethke.com.

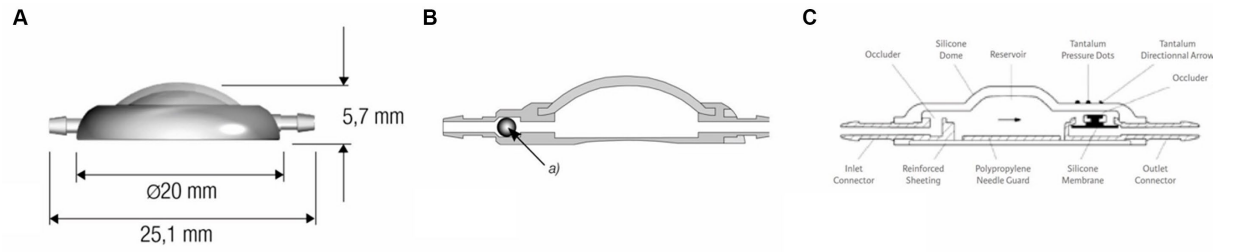

FIGURE 2
(A) Dome reservoir with titanium base and silicone dome, lateral view. **(B)** Control reservoir with non-return valve, technical drawing sagittal section (MIETHKE GmbH & Co. KG, Potsdam, Germany). Reprinted with permission Christoph Miethke GmbH & Co. KG; www.miethke.com. Technical drawing of the Contour-Flex™ Valve System (Integra Neuroscience) incorporating an internal diaphragma valve, which is mounted distal to an integral pumping reservoir.

infectious disorders (18, 21). Amongst other signs, MUOs result in seizures, which are relatively rarely seen in animals with hydrocephalus (5). In cats with internal hydrocephalus, CNS infection with feline infectious peritonitis (FIP) virus must be ruled out. Among other consequences, the virus causes ependymitis and choroid plexitis, which both impair CSF flow, such as through the mesencephalic aqueduct and out of the lateral apertures (24, 25). Although ependymitis and choroid plexitis are often detectable in MRI (24), imaging findings may be absent (25). Neutrophilic pleocytosis in CSF has a higher sensitivity for detecting FIP but may also be absent in some cases. Surgery should be planned only in absence of MRI and CSF findings that indicate inflammatory changes and with a negative RT-PCR result for FIP antigen obtained from CSF (25, 26).

The most common clinical signs associated with internal hydrocephalus are visual impairment, obtundation, ataxia, behavioral

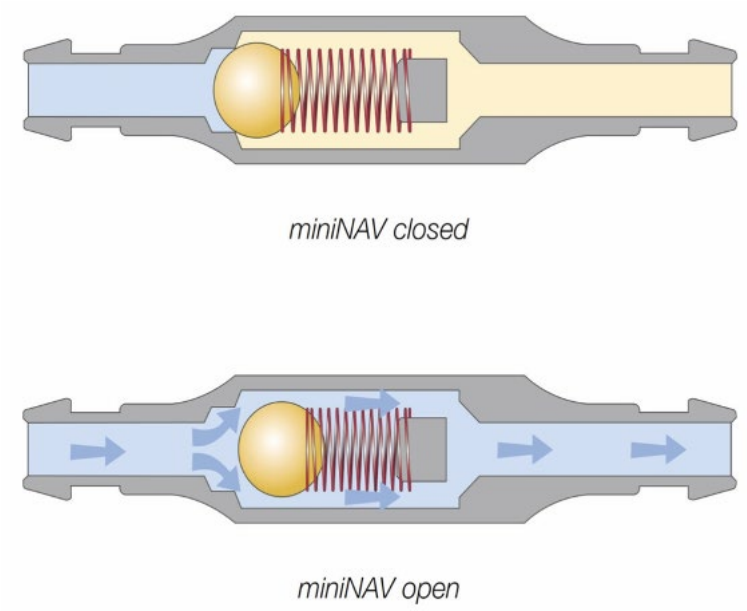

FIGURE 3
Differential pressure valve containing a ball in cone and spring valves (paediGAV®, MIETHKE GmbH & Co. KG, Potsdam, Germany). The titanium housing contains a spring fixed on a ball that blocks the passage. The resistance of the spring defines the opening pressure. If the intraventricular pressure exceeds the valve opening pressure, the spring force that otherwise holds the ball-cone valve closed is overcome. The spring is compressed, and the ball moves out of the cone, opening up a gap for fluid drainage. Permission granted by Christoph Miethke GmbH & Co. KG; www.miethke.com.

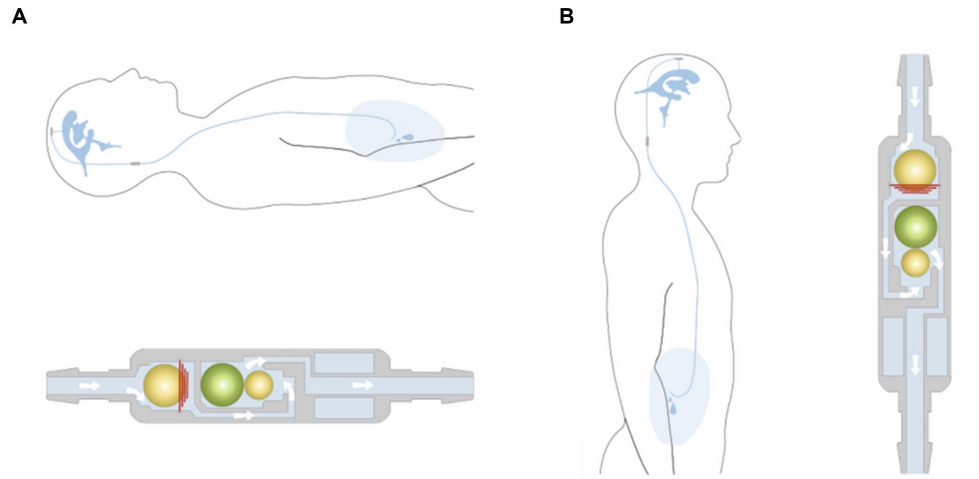

FIGURE 4
Example of a gravitational valve (GAV®; MIETHKE GmbH & Co. KG, Potsdam, Germany) that includes two valve chambers arranged in parallel. The first is a differential valve system with a prefixed opening pressure. In a horizontal position, this valve is the only one that resists flow. CSF flows without restriction through a second valve chamber. The gravitational unit starts working when the patient assumes an upright position. The postural changes move one or two balls from their original position to close the flow path in the second chamber. Permission granted by Christoph Miethke GmbH & Co. KG; www.miethke.com.

changes (circling, aimless barking), and ventrolateral strabismus. Head tilt and nystagmus can be associated with distension of the fourth ventricle (10). In addition to the association with classical clinical signs, MRI signs indicative of increased IVP might be helpful to diagnose clinically relevant internal hydrocephalus (16, 27). The presence of tetraventricular hydrocephalus, periventricular edema, and signal void sign in the mesencephalic aqueduct are indicative of IVP >12 mm HG (normal range 6–12 mm HG) (27). However, animals with hydrocephalus can have variable IVPs. IVP can be elevated, but also within normal ranges, and even below normal physiological values (9). The absence of raised IVP does not imply that the animal will not benefit from shunting (9). In summary, the

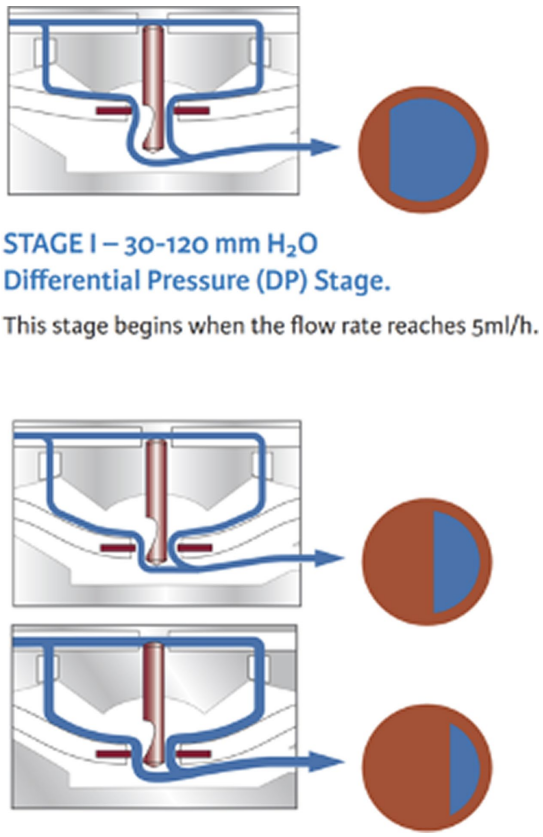

FIGURE 5
Example of a flow-regulating valve (Orbis Sigman valve; Integra neuroscience) that consists of a flow control rod that projects through an opening in a silicone membrane (flow orifice). The pin has a drop-shaped cut-out on its distal end that determines the diameter of the flow orifice and thereby the CSF volume flowing through the orifice. With increasing pressure, the membrane is deflected and creates a smaller orifice. Permission granted by Integra Life Science.

above-mentioned clinical signs and absence of inflammatory and other brain disease in association with ventricular distension are currently the best indicators that identify animals that will benefit from VPS.

Presurgical considerations

Dogs and cats with congenital internal hydrocephalus that are presented at a very young age of 2–4 weeks carry a high risk of anesthesia even for diagnostic imaging (28, 29–31). From experience, it is recommended to perform MRI and surgery at an age of at least 6 to 8 weeks minimum. If surgery is performed at such a young age, considerations must be taken on how much growth will be expected (e.g., Chihuahua vs. Rhodesian Ridgeback) to place enough loops for the shunt to expand.

Proper placement of the ventricular catheter at the largest point of ventricular enlargement is seeked (Figure 6). The ventricular catheter should be placed within the lateral ventricle with its tip ending at the level of the midline of the cerebral hemispheres to make sure a reconstitution of brain parenchyma is possible without obstruction of the catheter by the parenchyma. The depth of the aimed insertion length should therefore be measured on transverse MRI pictures before surgery.

As skull shapes vary among different brachy- and mesocephalic breeds, the ideal insertion point varies among individuals (32, 33). The aim is to place the ventricular catheter into the lateral ventricle but due to differences in skull shapes definite landmarks are hard to provide. Previous MRI might help to find the proper localization for shunt insertion and identification of some useful landmarks, e.g., temporal muscle lining, occipital protuberance, coronal sutures. Chihuahuas are often presented with internal hydrocephalus, but this breed is also common for large open fontanells and unossified sutures among the cranial vault (34, 35). This complicates proper shunt placement in these animals and might even make it impossible in rare cases.

Intraoperative IVP measurements are recommended to assess intraventricular pressure and choose an appropriate pressure valve system. Animals with an IVP of <5 mm Hg receive a differential pressure valve with an opening pressure of 5 cm H_2O, in animals with an IVP between 6 and 12 mm Hg receive valves with an opening pressure of 10 cm H_2O. In case of an IVP >13 mm Hg a 15 cm H_2O valve is commonly used (10). The opportunity to measure IVP is not available in most facilities, therefore a neurological examination and MRI interpretation might help to identify animals with normal IVP and those with high IVP to choose an appropriate low (5 cm H_2O) or high-pressure valve system (10–15 cm H_2O). Obtundation, nystagmus, and head tilt are clinical signs associated with increased IVP in dogs, whereas the involvement of all 4 ventricles, the presence of periventricular edema, and a T2-signal void sign within the ventricular spaces are associated MRI signs (27).

Concurrent disease like otitis externa or dermatitis are contraindications for surgery and should be treated and resolved before VPS placement to avoid infections.

Shunt-associated complications

Obstruction

A shunt is designed to stay implanted for a lifetime. However, shunt obstruction continues to be a common problem and might require replacement of various components. The silicone tube is a foreign material that can result in an intraventricular or intraparenchymal inflammatory reaction (36) (Figure 7). CSF proteins can adhere to shunt surfaces and promote cellular attachment and deposition of debris. Obstructions can occur for various reasons and may be divided into obstructions resulting from cellular debris or kinking of the catheter. Obstruction from cellular debris or parenchymal cellular infiltration occurs in about 10% of VPS (8).

In humans, obstruction can either occur in the ventricular catheter, inside the shunt valve, or in the peritoneal catheter. Shunt flow studies are routinely conducted to localize the obstruction site. By injecting a small volume of contrast agent into the shunt reservoir, the flow of CSF through the catheters and valve can be verified (37, 38). In our cohort of dogs and cats, the main site of obstruction was the ventricular catheter. Obstruction of the peritoneal catheter occurred in only one dog in our cohort, and the valve was never obstructed.

Correct positioning of the ventricular catheter is crucial to avoid catheter obstruction. The drainage segments must project freely into

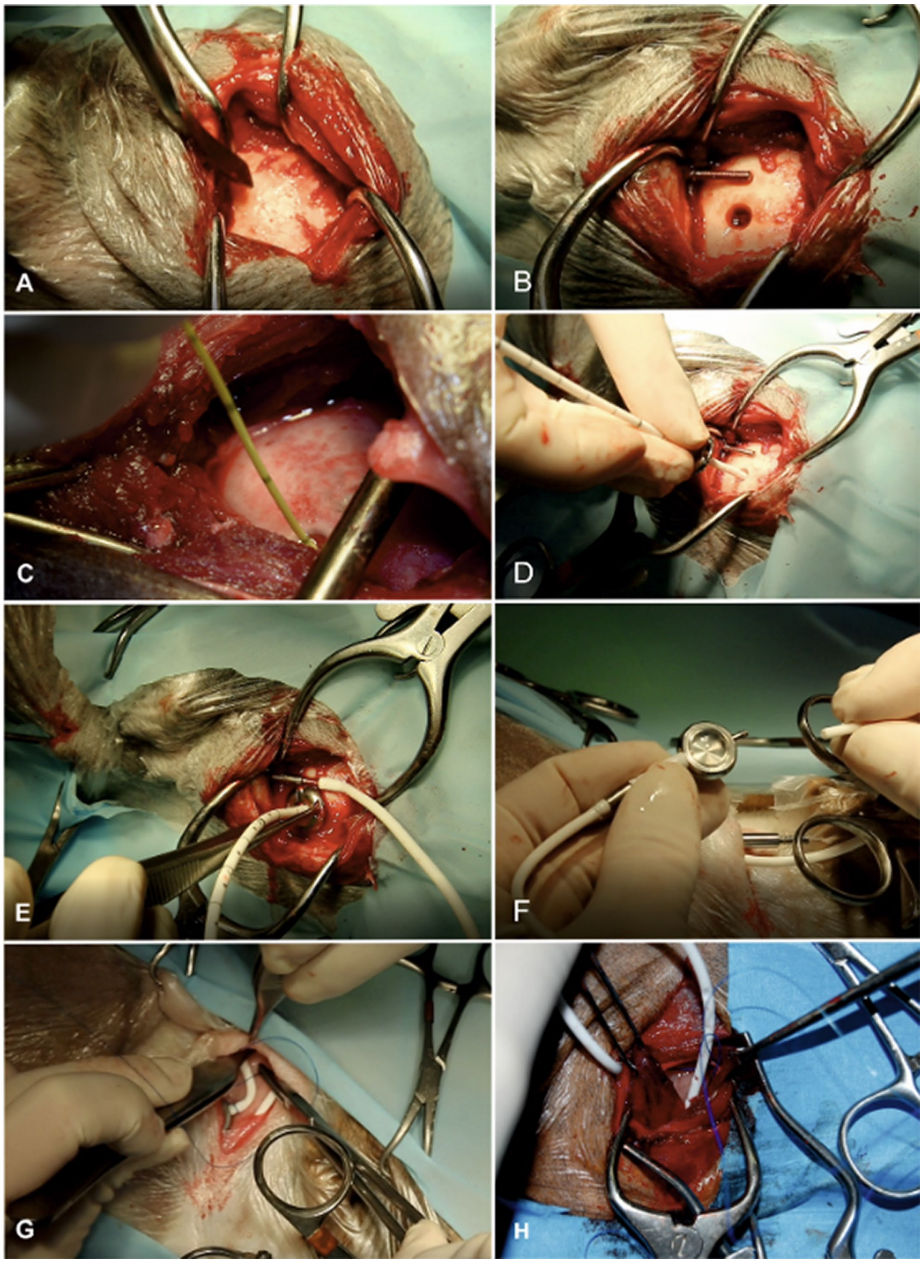

FIGURE 6
Surgical technique of ventriculo-peritoneal shunting (VPS). The hair on the lateral side of the cervical region, thorax, abdomen, and the whole head is clipped and aseptically prepared. Animals are placed in lateral recumbency. It is our aim to implant the shunt at the caudal horn to body transition where the dilation of the lateral ventricles is most pronounced, allowing for implantation with maximum depth. We also aim to position the shunt deflector underneath the masticatory muscles for protection and fixation. The most lateral point of the cranial vault (euryon) is palpated and the skull surface is exposed in this area **(A)**. A craniotomy hole is created in the skull with a pneumatic drill. The diameter must be adapted according to the diameter of the ventricular catheter. The dura is opened with a scalpel blade. Hemorrhage must be thoroughly controlled by bipolar cautery. A shunt passer is used to pass the distal end of the ventricular catheter underneath the masticatory muscles and then subcutaneously to the destination site in the lower dorsolateral cervical area. This needle must be placed before insertion of the ventricular catheter **(B)**. Intraventricular pressure is measured as a basis for the selection of an appropriate valve **(C)**. The cerebral cortex is then perforated with a stylet of a 20-gauge IV catheter. After punctuation, the ventricular catheter is inserted without resistance **(D)**. The tube is put in the deflector, which allows bending of the shunt without obliteration of its lumen. The distal end of the catheter is connected with the shunt passer and tunnelled under the muscles and skin towards the cervical incision **(E)**. The distal end of the ventricular catheter is connected to a CSF reservoir **(F)**. This dome is the centre for the S-shaped sling of the shunt tube. A narrow pocket is created craniodorsally and caudoventrally to the dome. After connection of the dome and the catheter parts, a semi-loop of the ventricular catheter is formed dorsally to the reservoir. The peritoneal catheter is connected and another semi loop is applied ventrally to the reservoir (or vice versa) **(G)**. These loops allow for stretching of the catheter system during head movements. The peritoneal catheter is again tunnelled under the skin towards the caudal aspect of the thoracic wall **(H)**. It is inserted in the peritoneal cavity via a small incision approximately 3 cm caudal to the last rib. Depending on the animal's size, the inserted tube length should be between 5 and 10 cm. Several holes are incised into the distal part of the abdominal catheter to allow CSF drainage along a long section of the catheter and to avoid obstruction of the tip by the abdominal momentum. The catheter is attached to the abdominal wall by a Chinese finger-trap suture. Closure of the abdominal wall is routine.

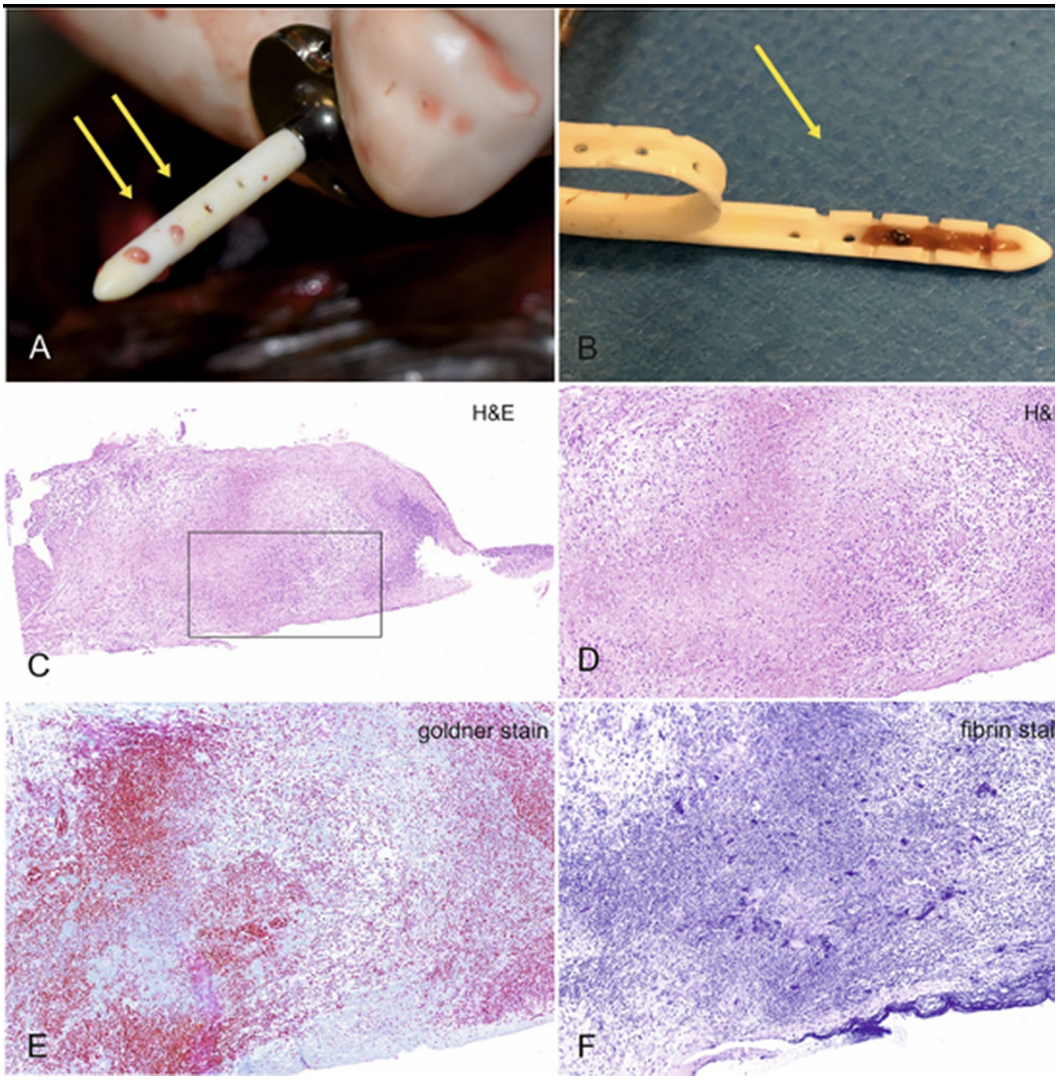

FIGURE 7
Typical appearance of a blocked shunt after removal from the ventricles **(A)**. After making a longitudinal cut through the catheter, blockage of the lumen within the first three drainage segments becomes obvious **(B)**. The last two segments were ensheathed by cerebral tissue and rendered useless. Histologic examination of the containing material revealed a mixture of granulation tissue with suppurative inflammation, erythrocytes, cellular debris, focal calcium deposits and siderophages, embedded in a collagen- and fibrin-rich stroma. **(C)** Overview, **(D)** 20× magnification, **(E)** Goldner stain for collagen, which is stained green-blue, and fibrin stain, which renders fibrin purple.

the ventricular space. Contact with the cerebral parenchyma must be avoided, as this increases the likelihood of ventricular catheter occlusion due to ingrowth of inflammatory cells into the catheter lumen (39, 40). The cerebral parenchyma usually re-expands in dogs and cats after successful shunting, resulting in encasement of the catheter and subsequent mechanical obstruction (2). It is therefore important to insert the catheter as deep as possible into the ventricles. In humans, the ventricular catheter needs to be positioned as far as possible away from the choroid plexus, which tends to block the catheter (41). The occipital horn is the most common location for implantation (41). Data on the influence of the insertion site of the catheter on the occurrence of catheter occlusion in animals are not available. Based on our experience, the choroid plexus is not involved in catheter obstruction in animals and special strategies to avoid placement of the catheter tip near the choroid plexus are not made (see Figures 5–7).

In the postoperative phase, the reservoir should be repeatedly pressed to confirm the refilling and patency of the ventricular catheter. After discharge, owners can use the reservoir to check if the shunt is patent. After compression of the dome it becomes concave and should re-inflate quickly to its previous convex shape, indicating that the shunt is refilling with CSF from the ventricles. A lack of reinflation is indicated by the dome keeping a concave shape after compression and indicates proximal obstruction of the ventricular catheter. If animals are readmitted due to lack of reinflation or recurrence of neurologic signs, radiographs of the entire course of the shunt tube must be obtained to rule out dislocation of the ventricular catheter, kinks, or disconnections in the shunt system. Absence of CSF after puncturing of the reservoir is another clear sign of a blocked ventricular catheter. If the ventricular catheter is blocked it needs replacement. Flushing of the ventricular catheter is not effective. CSF or cellular material taken from the removed catheter and CSF taken

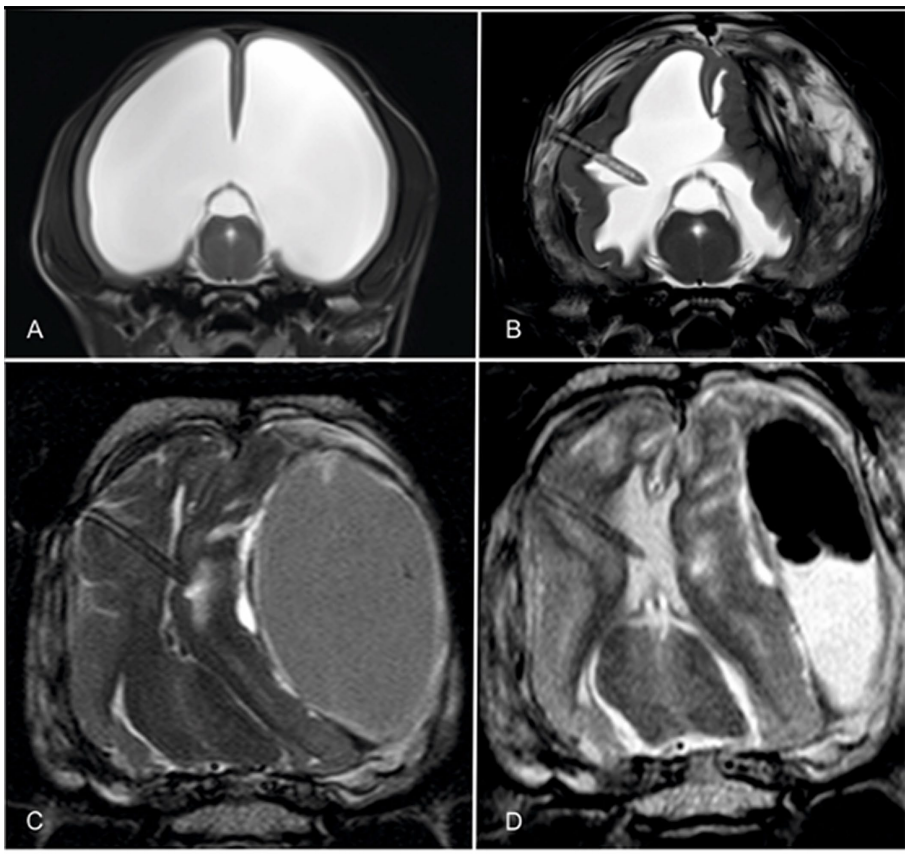

FIGURE 8
Transverse T2-weighted images through the brain of a hydrocephalic Chihuahua before **(A)** and after shunting and collapse of the right hemispheres and formation of an ipsilateral and contralateral hematoma **(B)**. The thickness of the hemisphere increased after shunting when the shunt was still working. Note the tissue that is being sucked towards the catheter tip. The ventral images show the brain of a mesocephalic mixed breed dog after implantation of a shunt. The ventricular space is totally diminished and the ventricular catheter is ensheathed with cerebral parenchyma **(C)**. After craniotomy and evacuation of the bulk of the hematoma, both the ventricles and the cerebral parenchyma partly re-expanded and the shunt continued draining CSF **(D)**.

directly from the ventricle should be submitted separately for bacterial examination (see below). Even if the bacterial examination is negative, the use of corticosteroids to reduce the inflammatory reaction against the shunt must only be considered with caution, especially as its benefits are not yet proven.

Overshunting

Overshunting describes a condition in which too much CSF is drained out of the ventricle too quickly, resulting in intraventricular under-pressure and thus tensile stress on the brain tissue. This tensile stress pulls the surface of the cerebral cortex away from the cranial wall, causing small bridging veins to rupture. The consequence is a slowly expanding intra-arachnoidal hemorrhage (10). This accumulation of blood causes compression of the hemispheres and potentially hemispheric collapse (Figure 8). The prevalence of overshunting-related complications such as hemispheric collapse and subdural hematoma in animals was 18% in a recent study (10). Signs of ventricular collapse are peracute occurrence of neurological signs.

Hematoma formation can result in subtentorial and intraforaminal herniation of brain tissue. In severe cases, the hemisphere of one side invaginates into the contralateral ventricle, which is fatal. Biventricular hydrocephalus and high grades of ventricular distension (42) are risk factors for the development of hemispheric collapse (10). Craniotomy and evacuation of the hemorrhage are life-saving procedures, can restore ventricular drainage, and can also effectively improve clinical signs (10, 43).

The use of gravitational valves in animals to prevent overshunting is somewhat controversial. One of the unanswered questions is how gravity affects CSF drainage in quadrupeds. Due to differences in the body axis in humans and animals, the shunt tube runs relatively vertically in both the lying and standing positions. Based on the assumption that a sitting position increases hydrostatic pressure in the shunt tube, gravitational valves were used in hydrocephalic dogs, but could not effectively prevent overshunting (10).

Other causes of shunt failure

Infection is the second most common cause of VPS malfunction in humans. Reported shunt infection rates range from 5 to 18%, with children under 1 year of age having the highest infection rate (44). It was suggested that the association of young age and infection may be caused by immaturity of the immune defence including the skin barrier, as well as delayed wound healing, and the etiology of

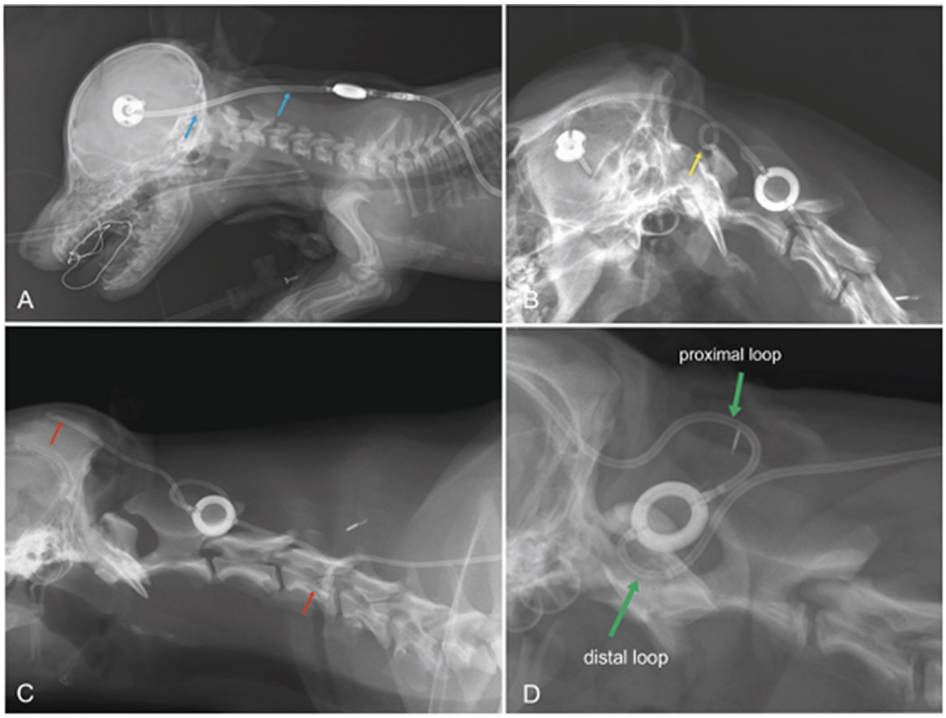

FIGURE 9
Laterolateral radiographs displaying the postoperative condition of dogs after VPS. In a Pinscher dog **(A)**, the distal end of the ventricular catheter is too straight (blue arrows). A sudden turn of the head and neck away from the implants will likely pull the catheter out of the cerebral ventricles. A tight expansion loop, as seen in **B** (boxer dog, yellow arrow), will likely result in transient kinking of the shunt on the ventral aspect of the loop, which might be permanent after the formation of scar tissue. The loop in **C** (Labrador retriever) was also not effective at preventing catheter migration (red arrow). In addition, there is a kink at the level of C5 (red arrow). A proximal and a distal half-loop is recommended (**D**; green arrows), as these can extend during stretching of the neck and there is no risk for coiling of the tube in the subcutaneous tissue (**D** Samoyed).

intraventricular hemorrhage (44). Compared to humans, infection is a relatively rare source of complication in dogs and cats (4.1%) (8). As shunt infection usually results in obstruction, recurrence of neurologic signs is also observed. As mentioned above, shunt infection does not necessarily result in brain infection. Depression, lethargy, craniospinal pain and fever are only observed if infection spreads to brain tissues, and not in shunt infection alone. MRI is indicated to reveal signs of encephalitis, meningitis and ventriculitis that need to be treated aggressively (45). Bacterial cultures taken from the shunt are mostly negative (8). In our cohort, *Staphylococcus epididermidis* and *Staphylococcus pseudintermedius* were the most common bacteria in positive cultures. In both encephalitis and pure shunt infection, removal of the infected shunt is indicated. The use of antibiotic-impregnated shunt tubes in humans is very effective at preventing recurrent infection/obstruction but not for treating encephalitis (Bactiseal® Integra Lifescience; XABO® MIETHKE) (46), as the antibiotic is only released into the shunt lumen. It is important to note that use of antibiotic agents in the tube (combination of rifampicin and clindamycin) may be restricted by European regulations governing antibiotic use in veterinary medicine.

Dislocation of the ventricular catheter and kinking of the subcutaneous tube are other rare complications. Occurrence of shunt dislocation can be reduced if expansion loops are placed underneath the skin, especially along the course of the tube that runs between ventricular catheter and reservoir. On the other hand, these loops are predisposed to kinking if not properly applied (Figure 9). However, especially in large dogs with long movable necks, or in puppies that underwent surgery at a very young age, shunt dislocation can be a serious and perennial problem.

Postoperative development and follow-up care

In primary/idiopathic hydrocephalus, neurologic dysfunction results from stretching of the periventricular white matter, as well as from impaired perfusion by periventricular blood vessels and impaired bulk flow of CSF within the brain tissue, leading to interstitial edema (18, 47, 48). VPS has the potential to reduce the ventricular dimensions, and allows parenchymal reconstitution. Based on our experience, signs of obtundation resolve very quickly within 12–24 h after surgery. Ataxia and cerebello-vestibular signs, on the other hand, take longer to improve (3–5 days). The presence of cerebello-vestibular signs in the first 2–3 weeks after surgery may raise the suspicion of a persistently dilated fourth ventricle, and a repeat CT/MRI is indicated. Based on our experience, the fourth ventricle is often not sufficiently drained, even if the dimensions of the lateral and third ventricle are decreased. The reason for this phenomenon is uncertain. Suboccipital craniectomy and incision of the caudal medullary velum can effectively remove CSF from the fourth ventricle, which results in permanent clinical improvement (unpublished data).

Visual deficits are in general hard to assess in animals and improvement of visual capabilities is likewise very difficult to determine. If destruction of the optic radiation is seen on preoperative MRI, central blindness will likely be permanent (2).

A regular follow-up neurological examination and repeat MRI is recommended after 3 months to evaluate clinical improvement, shunt function and possible reconstitution of brain parenchyma. If the animal has improved clinical signs and remains clinically unremarkable, there is actually no rationale for further regular follow-up. If the animal has not improved, it must be scheduled for re-examination, in which shunt patency must be checked (see below). If the shunt is patent and clinical signs have not improved, MRI should be considered. If ventricular volume has not decreased, implantation of a valve with a lower opening pressure should be considered. If ventricular size has decreased, and there is no clinical improvement, the lack of improvement must unfortunately be attributed to brain damage/atrophy that might persist after a decrease in ventricular volume (2).

Medical therapy

Medical therapy with prednisone, omeprazole, and acetazolamide to decrease CSF production by alteration of ion concentration and passive water current into the CSF space has been previously described for dogs and cats with congential internal hydrocephalus (48–50). But several studies describe no or just a limited effect of those drugs (6, 51–53).

Omeprazole was proven to reduce CSF production in experimental studies in dogs and rabbits if administered intravenously or intrathecally (54, 55). Oral administration (0.5–1.5 mg/kg) has therefore been proposed for long-term treatment of syringomyelia and internal hydrocephalus (1, 48, 49). However, oral dosages of 1 mg/kg are proven to be ineffective to lower CSF production in dogs (53).

The efficacy of glucocorticoids on CSF production has never been investigated and there is no evidence of efficacy for the treatment of internal hydrocephalus or syringomyelia in the current literature. Some studies however suggest their use for treatment of internal hydrocephalus and syringomyelia (48–50), but the effect is probably limited to the reduction of edema and inflammatory reactions rather than lowering CSF production (56).

Acetazolamide is the only drug with proven effect on CSF production by oral administration route in dogs and other species (52, 57–59). Nonetheless, acetazolamide is proven to be ineffective to reduce clinical sings and ventricular volume in a long-term follow up (6). Increased production of osmogenic ions from the ependyma and choroid plexus and an upregulation of acetazolamide-resistant isozymes occur after long-term use of acetazolamide. This could be the reason for the time limited effect on CSF production which lasts about 2-6 weeks (51, 52). It, however, may be used to lower CSF production for a few weeks, until a VPS surgery can be performed.

Alternative surgical procedures

Ventriculo-atrial shunts divert excess CSF into the internal jugular vein and further into the right atrium. An advantage of the procedure is the permanent intravenous pressure that resists CSF outflow if IVP falls below intravenous pressure, avoiding overshunting without a shunt valve. Ventriculosinusal shunts are other forms of vascular ventricular drainages that place the distal catheter in the sagittal-, or transverse sinus. The major disadvantage of these techniques is the risk of venous thrombosis and sepsis causing a high mortality (60). A plethora of alternative nonvascular ventricular drainages have been described including ventriculopleural shunt, ventriculo-gallbladder shunt, ventriculoureteral shunt, as well as drainage into the thoracic duct, or spinal epidural space. These techniques are used to avoid complications specific to VPS (i.e., intraperitoneal adherence, visceral perforations etc.) (61). Lumboperitoneal shunts (LPS) can be used to treat communicating hydrocephalus. VPS and LPS are equally effective to improve clinical signs in humans. The main advantage of the technique is that it is less invasive, avoids brain penetration and potential associated complications (parenchymal damage, subdural hemorrhage) (62). There is currently no experience with these techniques in veterinary medicine.

Procedures that do not use shunt systems are also scarcely described in hydrocephalic animals. Experience with ventriculo-subarachnoid fistulas, in which a connection between the ventricles and the subarachnoid space is produced, are limited to only few descriptions and their longterm outcome is uncertain (63).

Endoscopic third ventriclulostomy is a procedure mostly used to treat obstructive hydrocephalus in children (64). The procedure aims to create a passage between the third ventricle and the prepontine cistern. Again, there are no reports describing ETV in animals. When, or if, more evidence or experience is documented, these other techniques might be considered for the treatment of hydrocephalic animals in the future.

Conclusion

Surgical treatments for internal hydrocephalus continue to evolve. Refining patient selection and tailoring appropriate valves to the individual IVP is an important step in improving treatment. Proper placement of the ventricular catheter reduces the risk of catheter obstruction. Shunt disconnection and overshunting continue to be serious complications after VPS.

Author contributions

MS: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Validation, Visualization, Writing – original draft, Writing – review & editing. DF: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Validation, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Canine paroxysmal dyskinesia—a review

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Paroxysmal dyskinesias (PDs) are a group of involuntary, hyperkinetic movement disorders that recur episodically and may last seconds to hours. An important feature of PD is that there is no loss of consciousness during the episode. Using a clinical classification, three main types of PDs have been distinguished in canine PD: (1) paroxysmal kinesigenic dyskinesia (PKD) that commences after (sudden) movements, (2) paroxysmal non-kinesigenic dyskinesia (PNKD) not associated with exercise and can occur at rest, and (3) paroxysmal exertion-induced dyskinesia (PED) associated with fatigue. Canine PDs are diagnosed based on the clinical presentation, history, and phenomenology. For the latter, a video recording of the paroxysmal event is extremely useful. An etiological classification of canine PDs includes genetic (proven and suspected), reactive (drug-induced, toxic, metabolic, and dietary), structural (neoplasia, inflammatory, and other structural causes), and unknown causes. In this review, an overview of all reported canine PDs is provided with emphasis on phenotype, genotype, and, where possible, pathophysiology and treatment for each reported canine PD.

KEYWORDS

cramp, dyskinetic, hyperkinetic, movement disorder, dystonia

Introduction

Although it is likely that many movement disorders described in humans, also existed in dogs, the first report of a movement disorder in dogs dates back to 1969 (1). This is in sharp contrast with human literature. As of the 19th century, various movement disorders have been described. It is possible that Galileo Galilei was the first author who described, in the year 1,610, the existence of a physiologic tremor. Noteworthy is the publication of "shaking palsy" by Dr. James Parkinson in 1817 (2). Only when it became possible to record brief episodes of strange movements of our animals, first with video cameras and later with smartphones, both our awareness and interest in movement disorders grew, and several descriptions, of movement disorders in animals, have been published in the last decades.

The subject of this review is canine paroxysmal dyskinesia (PD). This is a group of involuntary, hyperkinetic, self-limiting, movement disorders that recur episodically and may last from seconds to hours. The other movement disorders, not subject to this review, are tremors, peripheral nerve hyperexcitability (3), and myoclonus (4).

Most PDs are the result of a dysfunction of the basal nuclei, although other central nervous system structures such as the cerebellum may also play a role (5–7). The neuroanatomy and neurophysiology of movement control were recently reviewed in the consensus statement of the International Veterinary Canine Dyskinesia Task Force (8).

PD has, in contrast with other movement disorders, a wide variety of clinical presentations (8, 9). An important feature is that during the episode, the dog remains conscious. An earlier review (9) classified canine PD according to the classification in humans (10). In analogy with human medicine, three main types of PDs have been distinguished in canine PD: (1) paroxysmal kinesigenic dyskinesia (PKD) that commences after (sudden) movements, (2) paroxysmal non-kinesigenic dyskinesia (PNKD) not associated with exercise and can occur at rest, and (3) paroxysmal exertion-induced dyskinesia (PED) associated with fatigue (9). In human medicine, the movements are described using words such as dystonia, ballism, chorea, athetosis, and choreoathetosis (10, 11). These terms cannot easily be extrapolated for use in our animals. For instance, athetosis is the involuntary contraction of (distal) limb muscles leading to a non-rhythmic bending/writhing movement of fingers, hands, or wrist (8, 10). Dogs cannot do this. Although there is great overlap in the anatomy of the central nervous system of humans and canines, there are differences that make it difficult to use the same terminology in both (5). For this reason, the recently published consensus statement was proposed to limit the terminology to be used, as there are distinct differences in anatomy and degrees of freedom of joint movement in humans and canines, preventing the use of words such as athetosis and chorea in our animals (8). Some of the used words, for instance, dystonia, have been used to describe a clinical diagnosis, whereas it is more logical to use it only as a clinical sign (12).

In this manuscript, we will use a classification based on etiology: genetic (proven and suspected), reactive (drug-induced, toxic, metabolic, and dietary), structural (neoplasia, inflammatory, and other structural causes), and unknown causes.

Diagnosis

When confronted with a possible movement disorder, it is important to describe in detail what is seen. The International Veterinary Canine Dyskinesia Task Force recommended categorizing all movement disorders into (1) hyperkinetic versus hypokinetic, (2) paroxysmal versus persistent, and (3) exercise-induced versus not related to exercise (8). A clear description of the clinical history and phenomenology may help to differentiate a PD from focal epilepsy and/or generalized epilepsy. In 2015, the International Veterinary Epilepsy Taskforce defined epileptic seizures as *"Manifestation(s) of excessive synchronous, usually self-limiting epileptic activity of neurons in the brain. This results in transient occurrence of signs which may be characterized by short episodes with convulsions of focal motor, autonomic, or behavioral features and due to abnormal excessive of synchronous epileptic neuronal activity in the brain"* (13). If available, an electroencephalogram (EEG) reading may help to differentiate a PD from (focal) epilepsy (14). However, EEG equipment is not readily available in routine clinical practice, and therefore, it is strongly recommended to document the clinical history and phenomenology and obtain video material of what is seen, but even then, it may be difficult to draw conclusions as demonstrated in a study investigating the inter-observer agreement of paroxysmal events and epilepsy (15). Paroxysmal dyskinesias are characterized by self-limiting episodes of abnormal movement. A PD does not cause pain, the dog is conscious, and there are no autonomic signs visible. A PD can last seconds, minutes, or hours, commencing and ending abruptly. In principle, there is no post-ictal phase, and a neurological examination between episodes is normal (9). However, dogs may be exhausted after a long-lasting episode.

Abbreviations: ASM, anti-seizure medication; CECS, canine epileptoid cramping syndrome; CKCS, Cavalier King Charles Spaniel; EEG, electroencephalogram; EMG, electromyogram; GTCS, generalized tonic–clonic seizure; GWAS, genome-wide association study; JRT, Jack Russell Terrier; PD, paroxysmal dyskinesia; PED, paroxysmal exertion-induced dyskinesia; PKD, paroxysmal kinesigenic dyskinesia; PKND, paroxysmal non-kinesigenic dyskinesia; SCWT, Soft-Coated Wheaten Terrier.

Genetic PDs

Cavalier King Charles Spaniel/episodic falling syndrome

Episodic falling syndrome (EFS) was first described in 1983 (16). Affected dogs present themselves with progressive hypertonicity of the limbs leading to a characteristic "deer-stalking" or "praying" position. Other clinical signs may include facial muscle stiffness, stumbling, a "bunny-hopping" gait, arching of the back, and vocalization (Supplementary Video S1) (17). The episodes, triggered by exercise, stress, apprehension, or excitement (16), last from less than 1 min to several minutes. It is the first PD in which the causative mutation was identified (17). It affects young dogs (up to 4 years) and is caused by a mutation in the brevican gene (BCAN). This gene encodes a brain-specific protein of the extracellular matrix proteoglycan complex. The abnormally formed protein results in a disrupted axonal conduction/synaptic stability (17, 18). The mode of inheritance is autosomal recessive. Although all CKCS fur color variants can be crossed among each other, EFS was predominantly seen in the Ruby and Black and Tan fur variants, as the mutated allele had a higher frequency in these fur colors. Interestingly, EFS improves with age which might suggest that in time compensatory pathways may lead to a resolution of the abnormally formed protein (9, 18). For this reason, if affected CKCS only have a low frequency of these episodes, the advice could be to avoid triggers and show some hesitance in medical treatment. Affected CKCS have been treated with both clonazepam and acetazolamide (17). This PD can be classified as a PNKD.

Soft-Coated Wheaten Terriers

The second PD in which the causative mutation was identified occurs in the Soft-Coated Wheaten Terrier (19). The authors identified 25 affected SCWTs with episodes of rapid flexion and extension of the hind limbs with varying degrees of truncal dystonia that lasted from several minutes to several hours and could occur as often as >10/day (19). Typical episodes consisted of the flexion alternating irregularly between limbs, but sometimes both hind limbs were off the ground simultaneously (Supplementary Video S2) (19). The authors reported that in severe episodes, the front limbs were also affected (19). The episodes were not associated with exercise but could be triggered by excitement and are classified as a PNKD. A mutation in the PIGN gene (autosomal recessive) is causative for the PNKD. The PIGN gene encodes an enzyme involved in the biosynthesis of glycosylphosphatidylinositol (GPI). This protein is involved in anchoring a variety of other proteins to the cell surface. In humans, mutations in this gene are associated with multiple congenital anomalies-hypotonia-seizures syndrome-1 (MCAHS1) but not with

a PD (19). Acetazolamide was tried in these dogs, and 11 dogs responded. In seven dogs, the episodes were completely abolished (20). Other treatments tried, with limited response, were clonazepam, levetiracetam, and phenobarbital (20).

Shetland Sheepdog

The third PD in which a genetic cause was associated with a PD was found in four female Shetland Sheepdogs. The clinical signs, mainly triggered by exercise and stress, consisted of a generalized ataxia with hypermetria and muscular hypertonia of all limbs, dystonia, normal to mildly reduced mentation, and a mild tremor (Supplementary Video S3) (21). By means of an organic acid analysis, a possible mitochondrial metabolic disease was identified. As three of the dogs were closely related (mother and two daughters), it was hypothesized to be an autosomal dominant mode of inheritance. Treatment with phenobarbital, diazepam, or levetiracetam did not resolve the clinical signs. However, a gluten-free, homemade fresh meat diet in three dogs or a tryptophan-rich, gluten-free, seafood-based diet appeared to alleviate the clinical signs (21). A genomic study revealed a case-specific missense variant in the PCK2 gene encoding the mitochondrial phosphoenolpyruvate carboxykinase 2. Sanger sequencing confirmed that all four cases carried the mutant allele in a heterozygous state (21). Several other affected female Shetland sheepdogs were identified by the first author of this manuscript (PM), and most cases responded to the dietary treatment.

Het Markiesje

In this small Dutch breed, several litters (approximately 10 to 12 weeks of age) were presented with clinical signs of severe PD (22). At rest, the pups were normal, but as soon as they started walking, severe tetraparesis, truncal and limb dystonia, cramping, and stumbling were observed (Supplementary Video S4). Treatment, with various medications (diazepam, phenobarbital, phenytoin, acetazolamide, and fluoxetine), was unsuccessful in all cases, and as the presentation deteriorated, elective euthanasia was performed (22). A pedigree analysis indicated an autosomal recessive mode of inheritance. Using a genome-wide association study (GWAS) and homozygosity mapping of 5 affected dogs from 3 litters, an associated locus on chromosome 31 in the region of SOD1 was identified. The DNA sequence analysis of SOD1 showed that the patients were homozygous for a frameshift mutation in the fourth codon, which means that the gene could not function. The findings were similar to a recent observation in human patients, where a loss-of-function mutation in SOD1 leads to a juvenile neurologic disease distinct from amyotrophic lateral sclerosis (22). This PD is classified as a PKD.

Weimaraner dogs

A recently described paroxysmal dyskinesia/dystonic ataxia was identified in Weimaraner dogs (23). The authors described four Weimaraners that were presented with episodes of an abnormal gait characterized as dystonic paroxysmal dyskinesia/ataxia. This could occasionally lead to collapse. Next to these signs, kyphosis and low head carriage were consistent features. The clinical signs were predominantly visible in the hind limbs although it could also affect the front limbs (Supplementary Video S5). The condition in these dogs deviates slightly from PD in that the dogs could have interictal signs and be abnormal for prolonged periods. Two dogs had intermittent anisocoria. This resembles episodic ataxia described in humans (24). The authors classified these episodes in the group of dystonia–ataxia syndromes (23). As written in the introduction, there are reports suggesting that in some conditions the cerebellum is involved as well (6, 7). Two cases responded well to fluoxetine treatment (23). A whole-genome sequencing revealed a private frameshift variant in the tenascin-R (TNR) gene in an affected dog leading to a non-functional protein. TNR is a member of the tenascin family of extracellular matrix glycoproteins (25). It is involved in neurite outgrowth and neural cell adhesion, proliferation and migration, axonal guidance, myelination, and synaptic plasticity (25, 26). It is exclusively expressed in the nervous system and is believed to be involved in a non-progressive neurodevelopmental disorder with spasticity and transient opisthotonos (26). The homozygotic variant was found in all four affected dogs and two unaffected heterozygotic Weimaraner dogs, but it was not found in a control group of 68 unaffected Weimaraners and 921 other dogs of various breeds (23). This PD could be classified as a PNKD.

Presumed hereditary

Scottish Terrier/Scottie Cramp

Scottie Cramp is the first reported PD in dogs (1). When first reported, it was an unknown neurological disorder. After telemetered EMGs, it was concluded that it was most likely a central nervous system disorder (27). It primarily affects dogs in the first year of life (6 weeks to 18 months), and bitches are overrepresented. Clinical signs can be triggered by excitement, stress, and exercise. These include generalized cramping or hind limb hypertonicity, and skipping and may last for 5 to 20 min (Supplementary Video S6). Avoiding triggers and behavior modification helped to reduce the number of episodes (28). In time, the episodes may decrease in severity and frequency. Fluoxetine reduced the episodes in some, but not all, cases. It is presumed to be autosomal recessive, but the fact that bitches are more affected than males contradicts simple Mendelian inheritance (28). Scottie Cramp is classified as a PNKD.

Chinook

Familial paroxysmal dyskinesia (PNKD) was identified in 16 of 51 Chinook dogs and is characterized by an inability to stand or ambulate, head tremors, and involuntary flexion of 1 or more limbs (Supplementary Video S7) (29). The episodes varied in duration from minutes to hours, and during these periods, the dogs remained conscious. In this breed, three dogs also had concurrent generalized tonic–clonic seizures (GTCS). It is believed to be autosomal recessive (29). Whether the GTCS and PD are associated in this breed is unclear. It is possible that a genetic pleiotropy exists between PD and epilepsy as recently reported in "Het Markiesje" (22) and humans (30), but like in the Labrador Retriever, Golden Retriever, and Labradoodle, PD and GTCS can co-exist in the same breed (31, 32). Hence, it is also possible that this is just coincidental. This PD is classified as a PNKD.

Jack Russell Terrier

The existence of PD in the Jack Russell Terrier (JRT) is reported in a study that describes the natural history of canine paroxysmal movement disorders in Labrador Retrievers and Jack Russell Terriers (32). Over a time period of 10 years, the authors examined 23 JRTs that were referred with clinical signs of PD (Supplementary Video S8).

Diagnosis was made based on the history, phenomenology, and video recordings (9, 32). The median age of onset in the JRTs was 4 years 8 months with 8 females (35%) and 15 males (65%). The episodes most commonly occurred following extremes of temperature in JRTs (32). The reported median frequency at presentation was one episode per month (range, one every 6 months to two per month). Two JRTs had cluster episodes (32). The purpose of this study was to describe the natural history if dogs did not receive treatment. During a follow-up time of over 3 years, five (22%) JRTs went into complete remission. JRTs that had a higher frequency (clusters of episodes) showed no remission. The authors concluded that treatment trials for canine PD should consider the natural history of this disease in untreated dogs before misattributing remission to specific treatment effects (32). This PD is classified as a PNKD.

Labrador Retriever—extreme muscular stiffness syndrome

In 2011, a novel hypertonicity syndrome in the Labrador Retriever was described (33). Although it is not a PD, it is reported in this review as next to this syndrome, PD exists in the Labrador Retriever as well (32–34). All reported cases were purebred male Labrador Retrievers. They were all young male dogs (15–47 months). The clinical signs were present for 2 to 16 months. Clinical signs consisted of continuous stiffness. It starts in the pelvic limbs and progresses to the thoracic limbs (Supplementary Video S9) (33). The dogs were treated, unsuccessfully, with various medications. Only non-steroidal anti-inflammatory drugs gave some improvement. The outcome was poor in all dogs. Four were euthanatized because of poor quality of life, two died of unrelated diseases. Only one dog remained alive, affected but stable (33). Necropsy performed in two dogs revealed astrocytosis throughout the spinal cord gray matter, reticular formation, and caudate nuclei. The disease is believed to be hereditary (X-linked).

Labrador Retriever—PD

The existence of PD in the Labrador Retriever is described in the already cited study that describes the natural history of canine paroxysmal movement disorders in Labrador Retrievers and Jack Russell Terriers (32). Over a time period of 10 years, the authors examined 36 Labrador Retrievers with clinical signs of PD. Diagnosis was made based on the history, phenomenology, and video recordings (9, 32). The clinical signs included dystonic hypertonia of one to all limbs, lifting one leg while lying, and inability to walk. When standing, the dogs could show hypertonia of all limbs (Supplementary Video S10). The median age of onset was 2 years and 3 months. The number of males is overrepresented [29 males (81%) and 7 females (19%)]. The episodes started after sudden movements or with excitement. The reported median frequency at presentation was once every 3 weeks. During a follow-up time of over 3 years, the frequency and duration of episodes decreased in 25 out of 36 (86%) Labradors, progressed in 5 Labradors, and remained static in 6 Labradors at follow-up (32). This PD is believed to be hereditary, and currently, a genetic study is being performed (34). This PD is classified as a PNKD.

Norwich Terrier

In 1984, the first report was published of an episodic muscular hypertonicity in the Norwich Terrier dog (35). In 2016, De Risio et al. (36) conducted a survey among Norwich Terrier owners. Twenty-six out of a group of 198 Norwich Terrier dogs (13%), using the questionnaire, video recordings, medical records, and telephone interviews, were classified as affected by PD (Supplementary Video S11). There was no sex predisposition. Between the episodes, all dogs were neurologically normal. The mean age at the first episode was 3 years. The episodes were characterized by sustained muscular hypertonicity in the pelvic limbs, lumbar region, and thoracic limbs, impairing posture, and locomotion without loss of consciousness (36). The episode frequency varied, and episodes lasted from 2 to 30 min. In 13 dogs, stress, anxiety, and excitement were identified as triggers. Various diets (gluten-free or meat-based) as well as different medications were tried. In none of the cases, clinical signs completely resolved with diets or medications alone (36). A pedigree analysis suggested that this PD is possibly hereditary (autosomal recessive) (36). This PD is classified as a PNKD.

Maltese dog

A recently performed retrospective study describes paroxysmal dyskinesia in the Maltese dog (37). The authors observed that in a group of 125 Maltese dogs, 19 had clinical signs suggestive of PD (15%). The dogs showed a combination of sudden dystonia of one or more limbs and generalized body tremors without loss of consciousness (Supplementary Video S12) (37). The episodes could occur after stress or exercise or during rest or sleep. The frequency varied from once daily to once per month or even less. The episodes lasted from 1 to 90 min with an average of 4.5 min. The mean age of clinical onset was 5.4 years. Acetazolamide was administered to six dogs, of which four responded partially. One responded to fluoxetine. Six out of seven dogs responded to a gluten-free diet. In two dogs, the PD resolved by itself. Although the authors did not perform a pedigree analysis, a hereditary cause is suspected (37). This PD is classified as a PNKD.

Welsh Terrier

In 2021, a case report described a levetiracetam-responsive PD in a Welsh Terrier. A 5.5-year-old, spayed, female Welsh Terrier dog exhibited, over a period of 12 months, recurrent episodes of involuntary hyperkinetic movements, abnormal muscle tone, and contractions triggered by exercise. As the PD worsened, the dog was put on levetiracetam and responded (38). Triggered by this report, another group of authors reviewed the clinical records of five referred Welsh Terriers with clinical signs of PD and performed a survey among Welsh Terrier owners. Clinical signs suggestive of PD were noted by 41 (22.8%) of 177 respondents (39). There was no sex predisposition. The median age of onset was 59 months. The episodes were characterized by sustained hypertonicity with periods of limb flexion, and abnormal head and body posture, without loss of consciousness (Supplementary Video S13) (39). The episodes varied in frequency and duration (from 30 s to 30 min). Various treatments, including gluten-free diets, with variable results, have been tried. Two additional dogs, in addition to the dog in the case report (38), responded to levetiracetam treatment (39). However, the PD was not progressive, and the frequency could decrease in time. Although no pedigree analysis was performed, a hereditary cause is suspected (39) (see Table 1).

Reactive causes

Drug-induced movement disorders are, in human medicine and most likely also veterinary medicine, a frequently observed, but not

TABLE 1 Overview of clinical characteristics of the genetic PDs.

Breed	Age	Clinical signs/ classification	Triggers	Cause	Duration	Progression	Treatment	Reference(s)
Genetic								
CKCS	Young dogs	"Deer-stalking" or "praying" position. Facial muscle stiffness, stumbling, a "bunny-hopping" gait, arching of the back, or vocalization (Supplementary Video S1) PNKD	exercise, stress, excitement	Autosomal recessive. BCAN gene	< 1 min to minutes. Low frequency possible.	Improvement over time	Expectative, clonazepam, and acetazolamide	(16, 17)
SCWT	Young dogs	Rapid flexion and extension of the hind limbs with varying degrees of truncal dystonia (Supplementary Video S2) PNKD	excitement, stress	Autosomal recessive. PIGN gene.	Several minutes to several hours. Several times a day.	Continuous	Acetazolamide	(19, 20)
Shetland Sheepdog	Young dogs to any age. females	Generalized ataxia with hypermetria and muscular hypertonia of all limbs (truncal), dystonia, normal to mildly reduced mentation, and a mild tremor (Supplementary Video S3)	excitement, stress, warmer weather	Dominant. Mitochondrial PCK2 Missense Variant.	Several minutes to several hours	Progressive	A fresh meat diet with supplements	(21)
Markiesje	Pups 10–12 weeks	Generalized stiffness, varying degrees of truncal and limb dystonia, and stumbling (Supplementary Video S4) PKD	exercise, walking	Autosomal recessive. SOD1 gene.	Continuous. Only stopped at rest.	Progressive	None	(22)
Weimaraner dogs	Young dogs	Dystonic gait/ataxia. Collapse, kyphosis, low head carriage. Predominantly hind limbs, sometimes also front limbs (Supplementary Video S5) PNKD	emotional arousal or exercise	Autosomal recessive. TNR gene	5 to 15 min. Several times a day possible.	Stable	Fluoxetine	(23)

always documented, cause for myoclonus, dystonia (tardive), dyskinesias, tremors, and/or parkinsonism (40). Antiseizure medications (ASMs) and antipsychotics are the most commonly reported drugs in humans (40). In veterinary medicine, the frequently used anti-emetic metoclopramide is known to induce extrapyramidal signs such as dystonia and dyskinesia when overdosed (41). In the central nervous system, the drug antagonizes dopamine D2 receptors, which most likely explains the extrapyramidal signs (41). Other known side effects are sedation, ataxia, agitation, nausea, vomiting, and constipation. Metoclopramide leaflets for human and veterinary use warn not to use the drug in patients suffering from seizures, tardive dyskinesia, and/or dystonia (42). Another frequently used medication is metronidazole. Metronidazole can cause a cerebellar/vestibular ataxia, nystagmus, paresis, and hypermetria (43–45). Most dogs will show clear cerebellar/vestibular ataxia, but the presence of the other neurological signs may confuse a clinician. The exact mechanism is unknown. It is possible that the inhibitory neurotransmitter gamma-aminobutyric acid receptor is modulated by the drug (45). Original reports document that prolonged use at high doses increases the risk of these side effects occurring, but a recent study showed that dogs can develop the side effects at lower doses and with shorter use as well (45).

In humans, several ASMs, among others phenytoin, gabapentin, and phenobarbital, have been associated with dyskinesias (41, 46). The exact mechanism is unknown. A postulated explanation was altered neurotransmitter levels (47), although a lowered folate concentration in blood has also been associated with prolonged use of phenobarbital (48). Phenobarbital can, at a high dose, cause various side effects in dogs such as lethargy, ataxia, paresis, and recumbency (42). In 2006, phenobarbital was reported to have induced PD in an epileptic Chow Chow (47). The dog developed twitches causing an inability to stand while on phenobarbital and bromide therapy. It was diagnosed as a PD, and when the phenobarbital dose was lowered, the dog returned to normal (47). The frequently used anesthetic induction agent, propofol, can also cause clinical signs of PD. Propofol decreases the

rate of dissociation of GABA from its receptors. Through prolonged binding, a chloride influx causes a hyperpolarization of the postsynaptic cell membrane (42). Propofol has been associated with PD/dystonia in humans (49, 50). Propofol is known to be able to cause myoclonus during anesthesia (Supplementary Video S14), which is thought to be caused by an imbalance of cholinergic-dopaminergic neurotransmitters (51). In 2013, a Goldendoodle showed dystonia of the neck and thoracic limbs in the recovery phase of anesthesia which included the use of propofol. As the effect of the propofol wore off, the dog became ambulatory again (51). In 2020, a crossbreed dog developed excitatory signs consisting of intermittent opisthotonic posture, limb dystonia, myoclonic jerks, paddling movements of all limbs, oculogyric movements, and excessive vocalization in the recovery phase of anesthesia induced by alfaxalone (52). This was successfully treated with the H1 antihistamine chlorphenamine (52).

The general conclusion is that signs of PD can occur if a dog receives certain medication or when it has ingested a drug or toxin that may have effects on the central nervous system. The advice is to search for known side effects and/or the mode of action. Variables such as genetic predisposition, environment, and age can play a role in the pathophysiology of these conditions and may facilitate side effects when a medication is given at a normal to higher dose (41).

Dietary causes

The influence of nutrients or supplements as an aid in the treatment of movement disorders has been examined in several studies. Subjects of study in humans have included, among others, essential fatty acids (53), dietary protein (54), the use of a ketogenic diet (55), gluten (56–59), supplements (60), and recently the role of the microbiome (gut–brain axis) (61).

In this review, the possible effect of a gluten-free diet or other types of diets in a few breed-related PDs has been raised by the authors. The PD observed in the Shetland Sheepdog appeared to respond to a homemade fresh gluten-free meat diet (21). Some of the Maltese dogs responded favorably to a gluten-free diet (37), and the same was noted for the Welsh Terrier (39). The only breed in which it has been thoroughly examined and a clear relation established is the Border Terrier (BT).

Border Terrier

PD in the BT was first named "Spike's disease" after the first dog, a Dutch BT named Spike, was diagnosed with a PD. In 2014, it was named Canine Epileptoid Cramping Syndrome (CECS) (62) although the authors at that time already classified it as a PD. In this first study, the authors described 29 young Border Terriers with clinical signs of difficulty in walking, a mild tremor, and dystonia (Supplementary Video S15). The episodes, lasting from minutes to hours, affected all four limbs as well as the head and neck in most cases. At that time, half of these dogs also developed gastro-intestinal complaints (vomiting and diarrhea), and approximately half of the dogs put on a gluten-free diet responded (62). In 2015, anti-transglutaminase 2 (TG2 IgA) and anti-gliadin (AGA IgG) were found to be elevated in 6 BTs with PD, all of which responded well to a gluten-free diet (63). Consequently, the PD was named a paroxysmal gluten-sensitive dyskinesia (9, 63, 64). In 2018, the same group measured TG2 IGA and AGA IgG in 128 BT and found that the results were conclusive to name it a gluten-sensitive PD (65). The clinical manifestation of PD in this breed has been described in 2016 in more detail (66) and attempts to unravel a genetic cause have been made in 2017 (67). The study of Stassen et al. (67) included 10 BTs in which EEG recordings were made. None of the recordings were abnormal strengthening the conclusion that it is indeed a PD (67). However, a GWAS did not identify significantly associated chromosome regions although a hereditary cause is suspected (67), but PD in the BTs is, without doubt, a gluten-sensitive PD. Gluten sensitivity, as a potential cause for neurological diseases in humans has been recognized for decades (56–59). Clinical manifestations include cerebellar ataxia (56) and PD (57), with associations postulated for conditions such as gluten encephalopathy, multiple sclerosis, peripheral neuropathies, sensorineural hearing loss, and epilepsy (68). Based on such observations and studies, it is postulated that gluten induces an immune-mediated response resulting in an antibody cross-reactivity between antigenic epitopes. In the case of gluten ataxia, this involves Purkinje cells and gluten peptides (56). In the BT, no studies, so far, have been performed investigating this possible pathogenesis in a gluten-sensitive PD (see Table 2).

Structural causes

Structural intra-cranial disease is also a differential diagnosis, although the exact link is unclear. It is possible that it is just a coincidental finding or that the structural cause triggers an existing PD. Earlier, we reported a secondary PNKD in a dog with multifocal forebrain lesions (9). This dog, diagnosed with meningoencephalitis of unknown origin, showed clinical signs of PD. When treated with prednisolone and cytosine arabinoside, the dog improved and showed no further signs of PD (9). A recent study investigating 100 dogs diagnosed with a head tremor demonstrated that this can be associated with an intracranial lesion in the thalamic region (69).

Unknown causes

Boxer pups

In two litters, a number of pups were identified with clinical signs described at that time as episodic involuntary skeletal muscle activity similar to human paroxysmal dystonic choreoathetosis, with normal levels of consciousness. The episodes lasted from 1 to 5 min. The affected dogs were presented between 5 and 9 months of age. Males were more frequently more severely affected. Next to the dystonic limb movements, the dogs could show unilateral facial dystonia or twitching. Except for some dogs that did not improve, almost all affected dogs did improve in time. Excitement was identified as a trigger. As it only occurred in two litters and not in other pups from the same parents, a genetic cause was excluded. The hypothesis was that it was either an acquired PD or an idiopathic PD (70).

Next, to these larger descriptive studies, there are a number of more anecdotal reports: a possible paroxysmal dyskinesia in two young Dalmatian dogs (71), Bichon Frise (72, 73), German Shorthaired Pointer (Supplementary Video S16) (74), and Golden Retriever (75), and the authors of this manuscript have identified PD in several other breeds such as the Australian Labradoodle (Supplementary Video S17), Basenji (Supplementary Video S18), Chihuahua (Supplementary Video S19),

TABLE 2 Overview of clinical characteristics of the presumed heritable PDs.

Breed	Age	Clinical signs/ classification	Triggers	Cause	Duration	Progression	Treatment	Reference(s)
Presumed heritable								
Scottish Terrier	Young dogs. Females more affected.	Generalized cramping or hind limb hypertonicity and skipping (Supplementary Video S6)/ PNKD	exercise, stress, excitement	Presumed heritable.	5 to 20 min.	Improvement over time	Fluoxetine	(27, 28)
Chinook	Young to middle aged dogs	Inability to stand or ambulate, head tremors, and involuntary flexion of 1 or more limbs (Supplementary Video S7)/ PNKD	None	Autosomal recessive. Presumed heritable.	Minutes to hours	Static in time	Not reported	(29)
Jack Russell Terrier	All ages, males predominate	Dystonic hypertonia of all limbs and inability to walk or stand (Supplementary Video S8)/ PNKD	Warmer weather/ extremes in temperature	Unknown		Static in time	Stable, improves with age	(32)
Labrador Retriever—Stiff Muscle Syndrome	young male dogs	Continuous stiffness, first started in the pelvic limbs and progressed to the thoracic limbs. (Supplementary Video S9)	None	Hereditary, X-linked Unknown	Continuous	Deteriorate	None	(33)
Labrador Retriever—PD	Young dogs, males predominate	Dystonic hypertonia of all limbs, hypermetria, and inability to walk or stand (Supplementary Video S10)/ PNKD	exercise, stress, excitement	Unknown/ autosomal recessive	Several minutes to rarely hours	Static in time	Clonazepam and acetazolamide	(32)
Norwich Terrier	Average age 3 years	Sustained muscular hypertonicity pelvic limbs, lumbar region, and thoracic limbs, impairing posture, and locomotion (Supplementary Video S11)/ PNKD	stress, anxiety, and excitement	Autosomal recessive	2 to 30 min	Static in time	None	(35, 36)
Maltese dog	Young dogs	Sudden dystonia of ≥1 limbs and generalized body tremors (Supplementary Video S12)/ PNKD	stress, exercise but also during rest or sleep	Hereditary	1 to 90 min	Infrequently, static in time	Gluten-free diet/ Acetazolamide	(37)
Welsh Terrier	Average age 5 years	Sustained hypertonicity with periods of limb flexion, abnormal head and body posture (Supplementary Video S13)/ PNKD	exercise	Hereditary	30 s to 30 min	Improves in time	Levetiracetam	(38, 39)
Border Terrier	Young to average age	Sustained hypertonicity with periods of limb flexion, difficulty walking, mild tremor, abnormal head and body posture (Supplementary Video S15)/ PNKD	stress, exercise	Gluten sensitivity and possible hereditary	Minutes to hours	Static in time but improves with diet change	Improves with a gluten-free diet	(61–66)

cross-breeds (Supplementary Video S20) (76), Dutch Shepherd (Supplementary Video S21), Manchester Terrier (Supplementary Video S22), Pomeranian (77) (Supplementary Video S23), Pug dogs (Supplementary Video S24), Springer Spaniel (Supplementary Video S25), Dutch Stabijhoun (Supplementary Video S26), Tervueren Shepherd

(Supplementary Video S27), and Yorkshire Terrier (Supplementary Video S28).

Diagnostic approach

The first step is to document the clinical history and phenomenology and obtain video material of what is seen. A physical examination will, between the episodes of a dog with an "idiopathic" PD, be normal. If any abnormality is noted, the advice is to pursue this further. Metabolic disorders can sometimes induce clinical signs fitting with a PD (hypocalcemia) or mimic clinical signs suggestive of PD. For these reasons, a thorough hematologic and clinical chemistry blood examination is advised to exclude reactive causes such as hypocalcemia, hepato-encephalopathy, hypoglycemia, uremia, hypo and/or hypercalcemia, Addison's disease, hypothyroidism, hyperthyroidism, and disturbances in sodium/potassium levels. Toxic causes, either acute or chronic, should always be considered as a possible cause. Acute intoxications are often accompanied by gastrointestinal and/or respiratory signs. The next step to exclude structural causes is a forebrain MRI. Although it may be tempting to skip this step in a breed in which a PD already has been described, if the possibility exists, the advice is to perform an MRI. Even in young dogs, structural and (inherited) metabolic causes and/or storage disease can occur as has been demonstrated in dogs with epilepsy (78, 79). A detailed description of the diagnosis of metabolic, toxic, and structural forebrain disorders can be found elsewhere (80).

When available, an EEG may help in the differentiation between (focal) epilepsy and PD (10, 14). If a causative mutation has been described in a dog belonging to a specific breed, such a genetic test should always be performed. As this may have implications for the population, the owner is advised to report it to the breeder/breed club.

Treatment

When a diagnosis of PD is established, the next step is to discuss with the owner what to do. Treatment, for the published PDs, has been discussed in the previous section. Based on the published cases, it is of great importance to realize that PDs can be self-limiting in several breeds. Examples are the PDs of the CKCS (17), SCWT (20), Scottish Terrier (28), Welsh Terrier (39), JRT (32), and Labrador Retriever (32). Whether to act immediately or not has to be discussed with the owner on an individual basis. It may be prudent to first monitor the natural course of the disease and consider a period of observation of at least 2 to 3 months to assess the pattern/frequency of the episodes in that individual dog before considering a therapeutic trial, especially in breeds not reported to respond to a particular treatment or diet. If triggers are noted, it is advised to avoid these triggers whenever possible. A gluten-free diet has been tried in several breeds and appeared to have some effect in the Maltese dog (37) and Welsh Terrier (39), but its effectiveness has been proven only in the Border Terrier (63, 65). Gluten may play a role in individual cases, as a recent publication investigating the presence of TG2 IgA and gliadin IgG in various dogs with PD found elevated levels in half of the examined dogs (81). Hence, a general recommendation is to measure the presence of anti-transglutaminase and anti-deaminated gliadin and consider a gluten-free diet accordingly. The presence of such antibodies should, however, not be overinterpreted as definitive proof of a causative role of gluten in PD in every case.

As for medications used in the management of PD, the various studies cited demonstrate that there is no golden treatment. In humans, carbamazepine is the drug of choice for kinesigenic dyskinesias (10) and clonazepam for the non-kinesigenic forms (82). Acetazolamide has been used successfully in the CKCS with EFS (17) and tried with various responses in several other breeds. Anecdotal evidence includes the successful use of phenobarbital in one GSHP that responded well to this treatment (74). Interestingly, fluoxetine has been used successfully in the treatment of PD in the Weimaraner (23), but it is the observation of the authors that medical treatment often remains "trial and error".

Prognosis

PD may be refractory to medication but if it is an idiopathic, presumed heritable, or genetic PD, it is often non-progressive. Even "Het Markiesje" dogs did not die from the disease itself. The owners were forced to elect euthanasia as none of the dogs responded to medication and were unable to walk any distance (22). The same applies to a number of dogs described in the various cited studies. Only if the dog was refractory and its welfare and wellbeing were seriously compromised, euthanasia was chosen. The fact that some of the PDs, in time, improve even without treatment strengthens the advice to await the natural course of the PD before drastic choices are made (9, 28).

Conclusion

Several canine PDs have been identified, and their specific features have been discussed. In five breeds up to now, the CKCS, SCTW, Shetland Sheepdog, Markiesje, and Weimaraner, a genetic cause has been identified. In several other breeds, a possible heritability has been postulated. Gluten sensitivity, as a cause, may occur in several breeds but has only been established in the Border Terrier. Diagnosing PD may be a challenge, but a logical step-wise approach is vital. Treatment is not always necessary as some PDs resolve spontaneously over time. Medical treatment results vary, and evaluation of the effect of treatment should take into account the natural course of PDs, including the possibility of spontaneous remission.

Author contributions

PM: Conceptualization, Investigation, Writing – original draft, Writing – review & editing. KS: Writing – review & editing. ML: Supervision, Writing – review & editing. LG: Supervision, Writing – review & editing.

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Conflict of interest

ML was employed by Movement Referrals: Independent Veterinary Specialists. LG was employed by Vet Oracle Teleradiology.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

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Advancing the early detection of canine cognitive dysfunction syndrome with machine learning-enhanced blood-based biomarkers

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Up to half of the senior dogs suffer from canine cognitive dysfunction syndrome (CCDS), the diagnosis method relies on subjective questionnaires such as canine cognitive dysfunction rating (CCDR) scores. Therefore, the necessity of objective diagnosis is emerging. Here, we developed blood-based biomarkers for CCDS early detection. Blood samples from dogs with CCDR scores above 25 were analyzed, and the biomarkers retinol-binding protein 4 (RBP4), C-X-C-motif chemokine ligand 10 (CXCL10), and NADPH oxidase 4 (NOX4) were validated against neurodegenerative models. Lower biomarker levels were correlated with higher CCDR scores, indicating cognitive decline. Machine-learning analysis revealed the highest predictive accuracy when analyzing the combination of RBP4 and NOX4 using the support vector machine algorithm and confirmed potential diagnostic biomarkers. These results suggest that blood-based biomarkers can notably improve CCDS early detection and treatment, with implications for neurodegenerative disease management in both animals and humans.

KEYWORDS

biomarker, blood–brain barrier, canine cognitive dysfunction syndrome, CCD rating scale, C-X-C chemokine ligand 10, NADPH oxidase 4, retinol binding protein 4, machine learning

1 Introduction

Global birth rates and increased human lifespans have sparked a trend in companion animal (CA) ownership, leading to concerns regarding long-term healthcare and medical costs for aging animals (1, 2). With noticeable increases in elderly dog populations, canine cognitive dysfunction syndrome (CCDS) has emerged as a common neurodegenerative condition

associated with age (3, 4). In aged dogs, this syndrome shares symptoms reminiscent of Alzheimer's disease (AD), the most prevalent cause of dementia in humans (4–7). CCDS is characterized by pronounced behavioral changes, such as evident spatial disorientation, nuanced changes in social interactions, compromised adherence to established housetraining protocols, and clear shifts in circadian rhythms and overall activity levels (8, 9). These behavioral alterations, exacerbated by a decline in memory functionality and learning ability (10–12), markedly distress CA owners and present considerable challenges for veterinarians tasked with treating these animals. Senior dogs are particularly vulnerable to CCDS; however, an objective diagnosis and treatment are still lacking. The onset of CCDS often goes unnoticed by CA owners as they frequently overlook early behavioral changes in their dogs (4). Typically, canines aged >7 years begin to display progressive behavioral and cognitive alterations associated with CCDS (8, 13) with the likelihood of developing CCDS increasing considerably with age (10, 12, 14, 15). By the time most senior dogs are diagnosed with CCDS, the condition is usually considerably advanced (4, 13, 16).

In clinical settings, the canine cognitive dysfunction rating (CCDR) is commonly used to identify cognitive deterioration in aging dogs (17); however, existing rating scales for CCDS have practical limitations. The assessment criteria of these scales tend to measure the rate of cognitive decline or frequency of unusual behaviors, potentially lacking the precision required to detect early cognitive changes indicative of CCDS. Identifying these deficiencies at an early stage greatly improves the chances of successful treatment (15). Online or telephone evaluations are susceptible to subjective interpretations by CA owners, possibly leading to over- or underestimation of disease severity (10, 12, 14). Current diagnostic methods for CCDS involve physical and neurological examinations, blood tests (such as serum analysis and complete blood cell count) to identify other conditions with similar symptoms, and the completion of CCDS screening questionnaires by owners (18). Unfortunately, comprehensive clinical tools to evaluate cognitive function in elderly dogs are lacking. While advanced techniques, such as MRI, are ideal for identifying neurological issues and evaluating cognitive deficits, the costs and need for sedation often make veterinary neurologists rely on neurological assessments instead (18). Given the anatomical complexities of the brain, it is difficult to assess progressive pathological shifts directly. However, there is a considerable need for diagnostic procedures based on objective findings from readily available animal biological specimens in clinical settings. Hence, biomarker analysis of the blood and cerebrospinal fluid is expected to emerge as the primary diagnostic method for CCDS (3, 12, 19). However, most CA owners prefer to collect peripheral blood samples over cerebrospinal fluid samples. Although the history of research on CCDS diagnosis using blood analysis is relatively short, recent efforts to identify valid biomarkers have been notable (3, 4, 20–23). Intriguingly, unlike humans, senior dogs with CCDS exhibited negligible $A\beta_{1\text{-}42}$ levels and minimal amyloid accumulation in brain tissue (24, 25). Therefore, there is a growing demand for promising alternative biomarkers to detect CCDS. However, proteomic analysis of canine blood using commercially available antibodies presents a notable challenge. Given that most research tools are designed for laboratory animals, there is a considerable limitation to advancing the development of canine biomarkers. Therefore, our selection of biomarkers encompassed those previously validated by our research group (26, 27), along with common biomarkers identified within the proteome array present in the peripheral blood of APP/PS1 mice, a standard model for AD, and the MPTP-induced Parkinson's disease (PD) model. Using blood analysis, we identified three early biomarkers of CCDS. The biomarkers under study, including retinol-binding protein 4 (RBP4), C-X-C-motif chemokine ligand 10 (CXCL10), and a marker previously identified by our team (NADPH oxidase 4, NOX4), were rigorously validated (26, 27). While numerous studies have suggested the potential of these biomarkers as indicators of nervous system function (28–35), there has been no endeavor to analyze and interpret the results derived from both factors collectively. Comparative proteomic analysis of Alzheimer's and Parkinson's disease models in mice and subsequent enzyme-linked immunosorbent assay (ELISA) evaluations underpinned their potential as reliable early indicators of CCDS. Additionally, a machine-learning framework applied to the dataset not only confirmed the robustness of these biomarkers but also their predictive power in clinical applications.

This paper details the methodology and findings of this novel approach with the intention of substantiating blood-based biomarkers as indispensable tools for early CCDS detection. We anticipate that the insights gained from this research will not only enhance CCDS management in dogs, but also offer a translatable framework for addressing human neurodegenerative diseases, thereby enriching the discourse on comparative medicine.

2 Materials and methods

2.1 Animal study

2.1.1 APP/PS1 mouse Alzheimer's disease

Serum samples from APP/PS1 transgenic mice were obtained from Laboratory Animal Resources Bank (LAREB, Daegu, Korea) at the National Institute of Food and Drug Safety Evaluation. All experiments were conducted with the approval of the Daegu Gyeongbuk Medical Innovation Foundation (approval number: DGMIF 21111602–00).

2.1.2 MPTP-induced mouse Parkinson's disease

C57BL/6J mice ($n = 20$; 7–8 week-old males) were used for the experiments. MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, $n = 10$) was intraperitoneally administered (S47312; Selleck Chemical, Houston, TX, United States) dissolved in 0.9% saline at a dose of 30 mg/kg/day for 30 consecutive days. All experiments were conducted with the approval of the Institutional Animal Care and Use Committee (IACUC) of Soonchunhyang University (approval number: SCH23-0043).

2.1.3 Canine sample acquisition

Blood samples representing 85 canines were collected by partnering with veterinary clinics and requesting that iamdt Co., Ltd. collect the samples with the consent of the CA owners. Blood samples, collected from healthy dogs and dogs with documented cognitive impairment using CCDR scores, were placed in EDTA anticoagulant tubes and separated into plasma and blood cells after centrifugation. Within 1 h of collection, the plasma was transferred to cryogenic tubes and frozen at −80°C until delivery from the veterinary clinics to Soonchunhyang University. All the blood samples used here were reviewed and approved by the IACUC of Soonchunhyang University

TABLE 1 The canine cognitive dysfunction rating (CCDR) scale.

CCDR scale	Score				
	1	2	3	4	5
1. How often does your dog pace up and down, walk in circles and/or wander with no direction or purpose	Never	Once a month	Once a week	Once a day	>Once a day
2. How often does your dog stare blankly at the walls or floor?	Never	Once a month	Once a week	Once a day	>Once a day
3. How often does your dog get stuck behind objects and is unable to get around?	Never	Once a month	Once a week	Once a day	>Once a day
4. How often does your dog fail to recognize familiar people or pets?	Never	Once a month	Once a week	Once a day	>Once a day
5. How often does your dog walk into walls or doors?	Never	Once a month	Once a week	Once a day	>Once a day
6. How often does your dog walk away while, or avoid, being patted?	Never	Once a month	Once a week	Once a day	>Once a day
7. Compared with 6 months ago, does your dog now pace up and down, walk in circles and/or wander with no direction or purpose?	Much less	Slightly less	The same	Slightly more	Much more
8. Compared with 6 months ago, does your dog now stare blankly at the walls or floor?	Much less	Slightly less	The same	Slightly more	Much more
9. Compared with 6 months ago, does your dog urinate or defecate in an area it has previously kept clean? (if your dog has never house-soiled, tick 'the same')	Much less	Slightly less	The same	Slightly more	Much more
10. Compared with 6 months ago, does your dog fail to recognize familiar people or pets? (Multiply by 3)	Much less	Slightly less	The same	Slightly more	Much more
Total	12–24 = Normal 25–35 = MCI[1] >36 = SCI[2]				

[1] Mild cognitive impairment, [2] Severe cognitive impairment.

(approval number: SCH23-0069). Additionally, blood samples from beagle dogs (two aged 5 months and one aged 96 months) that had not undergone any surgical or chemical treatment were obtained from LAREB. The experiments were conducted with the approval of the Daegu Gyeongbuk Medical Innovation Foundation (approval numbers: DGMIF-21071203-00 for the beagle dogs aged 5 months and KMEDI-22071501-00 for the one aged 96 months).

2.1.4 Canine experimental groups

The animals were classified into four groups: normal; mild cognitive impairment (MCI), severe cognitive impairment (SCI), and CCDS. The grouping of categories MCI, SCI, and CCDS is based on the CCDR (12). Initially comprising 13 behavioral items, this scale was refined to 10 questions by excluding three questions that were commonly found to be challenging to answer (Table 1). The cumulative scores range from 0 to 60, with scores between 25 to 35 indicating MCI and scores >36 indicating SCI. Additionally, a CCDS group was incorporated into our classification, encompassing both MCI and SCI.

2.2 Proteome profiler arrays

The cytokines, chemokines, and growth factors present in the mouse serum from both the control and disease groups (AD or PD) were semi-quantitatively evaluated using the Proteome Profiler Mouse XL Cytokine Array Kit (ARY028; R&D Systems, Minneapolis, MN, United States). Serum samples were processed by incubation on nitrocellulose membranes, pre-spotted with capture antibodies in strict adherence to the manufacturer's instructions. Next, each membrane was treated with a biotinylated antibody cocktail, and subsequently with streptavidin bound to horseradish peroxidase (HRP). HRP luminescence was developed and positive signals were captured using a Chemi Reagent Mix on a light-sensitive X-ray film with exposure times ranging from 1 to 10 min. Quantification of the fold changes involved analysis of the expression levels of each spot on the membrane using HLImage++ Software (v25.0.0r, Western Vision Software, Salt Lake City, UT, United States), and the intensities were compared against the mean values of the control samples.

2.3 Antibodies

The following antibodies were used: monoclonal mouse anti-RBP4 antibody (orb751184, Biorbyt, Berkeley, CA, United States); polyclonal rabbit anti-CXCL10 antibody (abx104024, Abbexa, Cambridge, United Kingdom); polyclonal rabbit anti-NOX4 antibody (NB110-58849, Novusbio, Centennial, CO, United States); polyclonal rabbit anti-transferrin antibody (NBP1-97472, Novusbio, Centennial, CO, United States); peroxidase labeled horse anti-mouse IgG (H + L) (7076P2, Cell Signaling Technology, Danvers, MA, United States); peroxidase labeled goat anti-rabbit IgG (H + L) (PI-1000, Vector Laboratories, Burlingame, CA, United States).

2.4 Immunoblot

Canine plasma was homogenized in RIPA buffer (R0278, Sigma-Aldrich, St. Louis, MO, United States) and augmented with a phosphatase inhibitor (P3200, GenDEPOT, Barker, TX, United States). To ensure precise analysis of plasma components, a Minute™ albumin depletion reagent (WA-013, Invent Biotechnologies,

Plymouth, MN, United States) was employed, effectively removing albumin. The efficacy of this depletion was confirmed using SDS-PAGE and Coomassie Brilliant Blue staining (CR2006, Biosesang, Yongin, Korea). Protein concentrations in albumin-reduced plasma were quantified using a BCA assay kit (21,071, iNtRON Biotechnology, Seongnam, Korea). Following SDS-PAGE on a 10–15% tris-glycine gel, the proteins were transferred onto a PVDF membrane (10,600,023, GE Healthcare, Freiburg, Germany). Membranes were blocked using 5% bovine serum albumin (BSA, SM-BOV, GeneAll Biotechnology, Seoul, Korea) in 1X TBS-T (10X TBS with Tween 20, TR2007, Biosesang, Yongin, Korea). Primary antibodies—specifically monoclonal mouse anti-RBP4, polyclonal rabbit anti-CXCL10, and polyclonal rabbit anti-NOX4—were incubated overnight at 4°C in 1% BSA in TBS-T. HRP-conjugated secondary antibodies, horse anti-mouse, and goat anti-rabbit were then applied for 2 h at ambient temperature. Detection of target proteins was facilitated by ECL western blotting detection reagents, with signals visualized using a chemiluminescence bioimaging instrument (CELLGENTEK, Daejeon-si, Korea). Analytical assessment was performed using the ImageJ software v1.52t (Bethesda, MD, United States).

2.5 Enzyme-linked immunosorbent assay (ELISA) for the diagnosis

Blood samples were analyzed for RBP4 (MBS739348), CXCL10 (MBS747479), NOX4 (MBS737351), P-Tau (MBS7230007), and NfL (MBS7231454) using ELISA kits from MyBioSource (San Diego, CA, United States). Each well of a plate, pre-coated with specific detection antibodies from the respective kits, received canine plasma containing RBP4-, CXCL10-, NOX4-, P-Tau-, and NfL-HRP-conjugated antibodies. The reactions were performed at 37°C for 1 h. Following incubation, the plasma and antibodies were discarded, and each well was thoroughly washed with washing buffer. Subsequently, a substrate solution was dispensed into each well, and the plate incubated again at 37°C for 15 min under light-protective conditions. Upon the addition of the stop solution, a colorimetric change from blue to yellow was observed, and the optical densities of the resulting solutions were measured at 450 nm using a microplate reader.

2.6 Machine learning for CCDS speculation

Here, we analyzed the CCDS classification using proposed biomarkers for machine learning, for which we utilized Python (v3.6.13)'s scikit-learn (v0.24.2). In the machine-learning classification task, three comparisons were made: normal vs. MCI, normal vs. SCI, and normal vs. CCDS with the numbers of samples used in these classifications being 50, 46, and 85, respectively. The machine-learning model was trained and evaluated through 10 repeated experiments. Of the total data, 70% were used as training data and the remaining 30% as test data. The machine-learning model utilized various algorithms, including support vector machines (36), extra trees (37), random forests (38), gradient boosting (39), bagging (40), AdaBoost (41), and XGBoost (42), all of which provided by Scikit-Learn. Performance was measured using metrics such as Area Under the Curve (AUC), Accuracy, and the F1 Score.

2.7 Statistics

All statistical analyses were performed using Prism 10 (GraphPad Software Inc., San Diego, CA, United States). Data are presented as mean ± standard error of the mean (SEM). Statistical analyses were performed using a Student's two-tailed t-test to compare the two groups. For multiple group comparisons, an analysis of variance (ANOVA) with *post hoc* comparisons was performed using Tukey's multiple comparison test. p values (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; and **** $p < 0.0001$) were considered statistically significant.

3 Results

3.1 Novel biomarker screening in AD and PD models

Our study involved screening for new biomarkers within the proteomic profiles of blood samples from AD and PD mouse models as well as from normal animals (Figure 1A). Our results indicated that among the 111 inflammatory cytokine markers analyzed, RBP4 and CXCL10 showed significant differential expression in both AD and PD models. These findings suggest that RBP4 and CXCL10 in the blood could serve as reliable indicators of neurodegenerative diseases (Figure 1B).

3.2 RBP4, CXCL10, and NOX4 are decreased in the plasma of canine with cognitive decline

3.2.1 General details of the canines used

The general details of the animals used in this study are shown in Table 2.

3.2.2 Validation of the usefulness of selected biomarkers expressed in canine plasma

To validate the observed results for RBP4 and CXCL10 in the disease animal model, we conducted western blot analysis (Figure 2A). Moreover, we investigated the association between the expression of NOX4—a key molecule contributing to the progression of AD and PD in the brain as identified in previous studies—and the expression of RBP4 and CXCL10 in canines. We identified a significantly lower expression of RBP4, CXCL10, and NOX4 in the plasma of CCDS canines than in normal canines, consistent with the results obtained from APP/PS1 and MPTP-induced mice (Figure 2B).

3.2.3 Biomarker expression measurements in canine blood collected from outpatients using ELISA analysis

We confirmed the utility of the biomarkers by performing ELISA with additional samples (Figure 3). Similarly, decreased expression of RBP4, CXCL10, and NOX4 was observed in the plasma of CCDS dogs compared to normal dogs. Although the p value for RBP4 was not

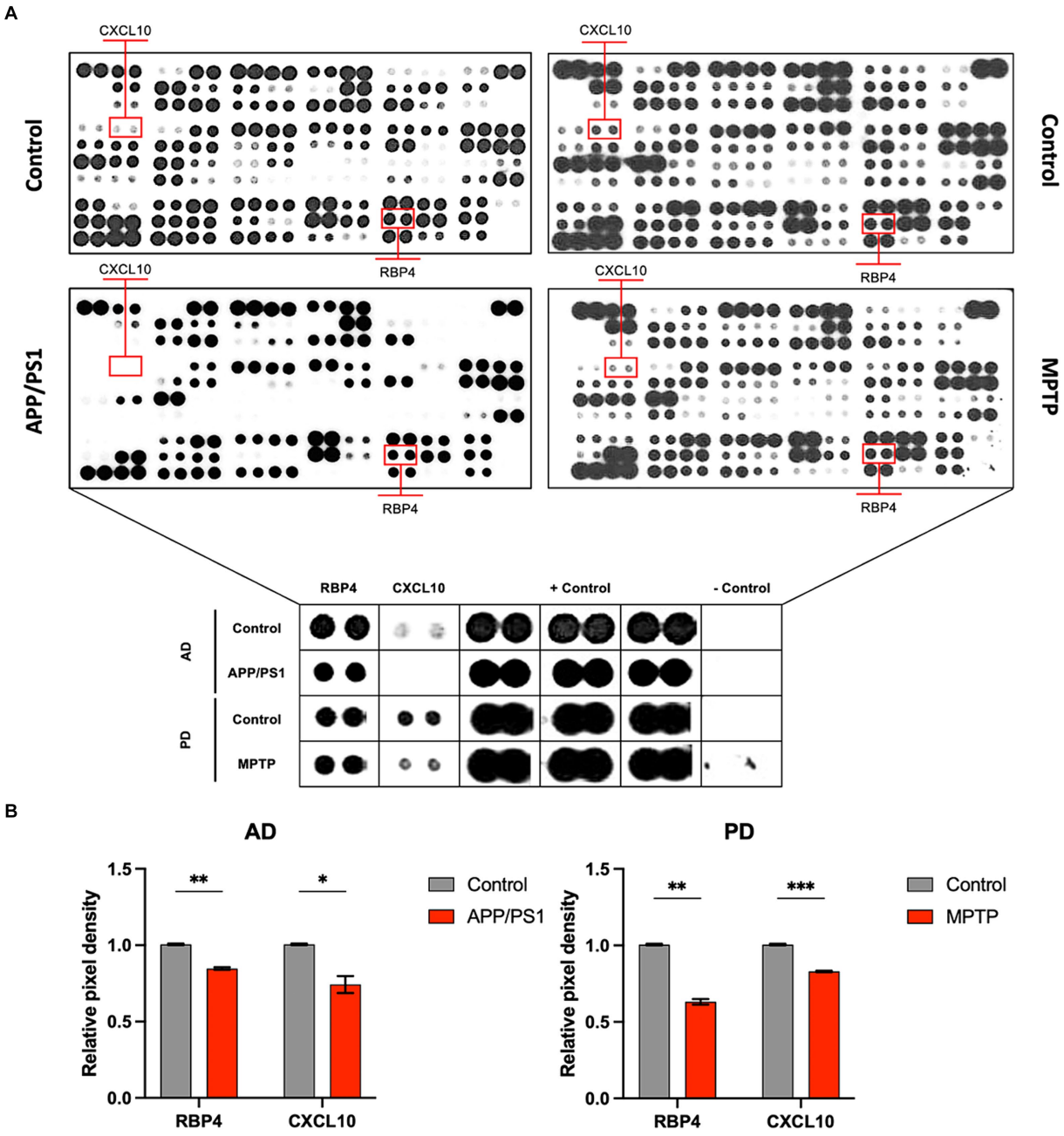

FIGURE 1
Protein profiling of the serum from APP/PS1 mice and MPTP-induced mice for disease model animals was obtained by performing proteome array analysis. **(A)** Images of array spots on the membrane are proteome array for immune blot, including 111 cytokine, chemokine, and growth factor types. The red rectangle indicates RBP4 and CXCL10 showing expression levels in the APP/PS1 and MPTP-induced mice. Magnifying indicates an enlarged image of RBP4 and CXCL10 in the square below each image. Array spots were analyzed according to the manufacturer's instructions. **(B)** Quantifying RBP4 and CXCL10 levels in control and disease model animals from serum. Data are mean ± standard error of the mean (SEM). *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$ vs. control by a Student's two-tailed t-test. AD: Alzheimer's disease; PD: Parkinson's disease.

TABLE 2 **Baseline characteristics of all canines in the study.**

Groups	Normal	MCI[1]	SCI[2]	CCDS[3]
N = (%)	37 (44)	25 (29)	23 (27)	48 (56)
Age, y (Mean ± SD)	7 ± 3.5	12 ± 3.0	15 ± 2.7	14 ± 3.3
Males, %	16 (43)	14 (56)	11 (48)	25 (52)
CCDR score (Mean ± SD)	24 ± 0.0	30 ± 4.0	49 ± 7.5	40 ± 11.4

[1] Mild cognitive impairment, [2] Severe cognitive impairment, [3] Canine cognitive dysfunction syndrome.

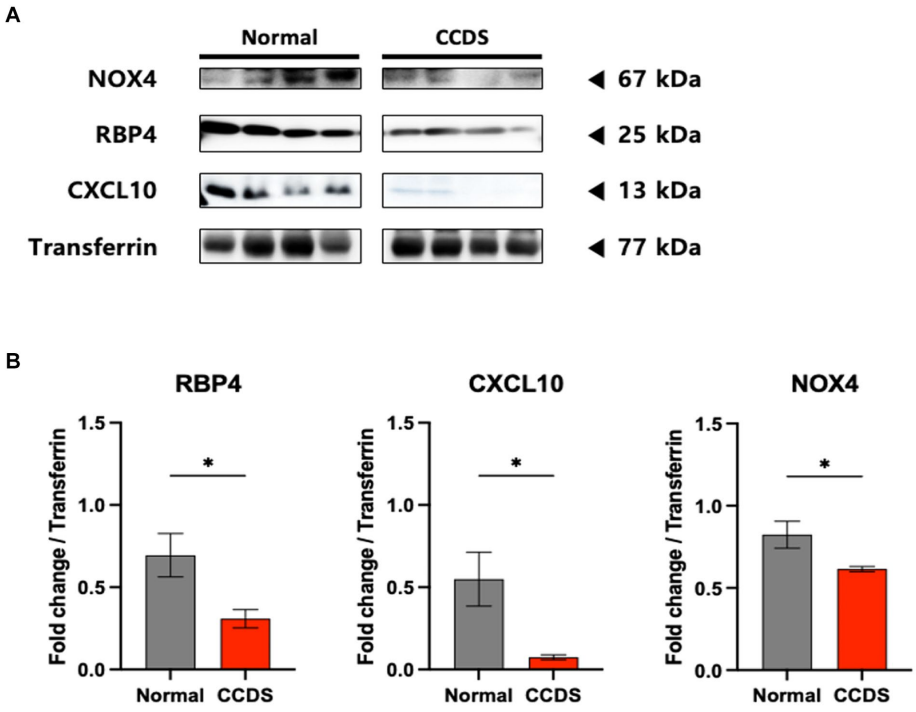

FIGURE 2
RBP4, CXCL10, and NOX4 protein levels in dogs of normal and CCDS groups. **(A)** Representative immunoblot analysis of RBP4, CXCL10, and NOX4 expression in CCDS groups compared to normal groups. **(B)** Quantifying RBP4, CXCL10, and NOX4 levels in normal and CCDS groups from plasma. Data are mean ± SEM. *, $p < 0.05$ vs. normal by a Student's two-tailed *t*-test.

significant, a declining trend was noted in the concentrations between normal and CCDS dogs. Notably, in comparison to the normal group, significant decreases in CXCL10 and NOX4 were observed in the MCI and SCI groups, respectively. Likewise, when compared to the combined MCI and SCI group (CCDS group), the measurement values of normal animals and statistical significance were also established. This observation is consistent with the results found in mouse models of the disease.

3.3 Machine learning for the CCDS classification

This study applied machine-learning algorithms to evaluate the combination of biomarkers that would most effectively determine a dog's condition (normal, MCI, SCI, and CCDS). Changing the number of samples used in machine learning can notably affect the results. Therefore, the analysis was performed on an equal number of randomly selected samples in order to equalize the number of normal samples in the analysis group. Ten randomly repeated experiments were performed using seven classification algorithms, with 70% of the total data used as training data in each experiment.

3.3.1 Identification of correlations between variables

Prior to the development of the machine-learning model, a correlation heatmap was generated to assess the relationships between the variables using all 85 samples (Figure 4). The variables analyzed included RBP4, CXCL10, NOX4, CCDR, and age. Higher and lower correlations are depicted in red and blue, respectively. Notably, the correlation coefficient between CCDR and age was 0.66, indicating an increasing trend in the CCDR scores with advancing age. A significant correlation ($r = 0.7$) was observed between CXCL10 and NOX4. Furthermore, the results for RBP4, CXCL10, and NOX4 appeared to be inversely proportional to the CCDR scores, with their levels diminishing as CCDR scores increased.

3.3.2 Normal and MCI state prediction

To assess the discriminative ability between normal and MCI, we randomly selected an equal number of normal samples for analysis, matching the 25 MCI samples. Among the biomarker combinations listed in Table 3, the RBP4 and NOX4 combination yielded the highest prediction results, with an F1 score of 0.84. However, the other three combinations resulted in an F1 score of 0.77 (RBP4 + CXCL10 & CXCL10 + NOX4) or 0.73 (RBP4 + CXCL10 + NOX4). In the comparative analysis between normal and MCI samples, the gene combination of RBP4 and NOX4 emerged as the most effective among all combinations (Table 3). Utilization of the receiver operating characteristic (ROC) curve for predicting favorable outcomes in the normal and MCI states was based on animal plasma analysis (Figure 5A).

3.3.3 Normal and SCI state prediction

When comparing the results of the three combinations formed by pairing two genes, that of RBP4 and NOX4 exhibited the highest performance (F1 score of 0.73), while that of RBP4 and CXCL10 yielded the lowest result (F1 score of 0.59) (Table 4). Additionally, when all three genes were used, the Extra Tree showed the best

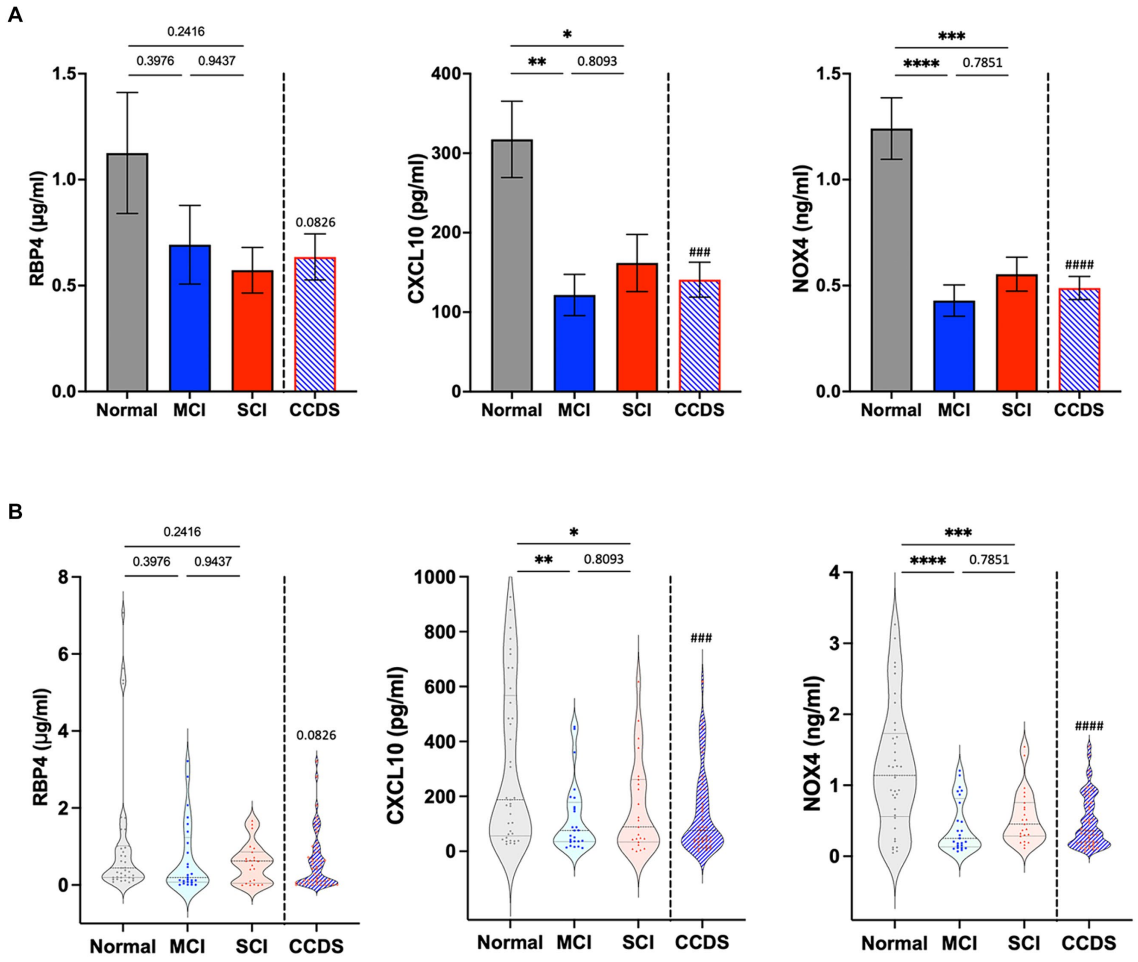

FIGURE 3
RBP4, CXCL10, and NOX4 levels were measured in the plasma from animal groups diagnosed with mild cognitive impairment (MCI) and severe cognitive impairment (SCI) based on CCDR scores. CCDS represents a group inclusive of both MCI and SCI, separated by a dotted line. **(A)** The bar graphs represent the quantification of RBP4, CXCL10, and NOX4 ELISA levels in each group. Data are mean ± SEM. **(B)** Representative violin plot graphs of the distribution of biomarker levels in each group. The dot in the graph revealed the distribution of individual samples, and the lines in the violin shape represent quartiles and medians. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$ vs. each group by a one-way ANOVA. ###, $p < 0.001$; ####, $p < 0.0001$ vs. normal by a Student's two-tailed *t*-test.

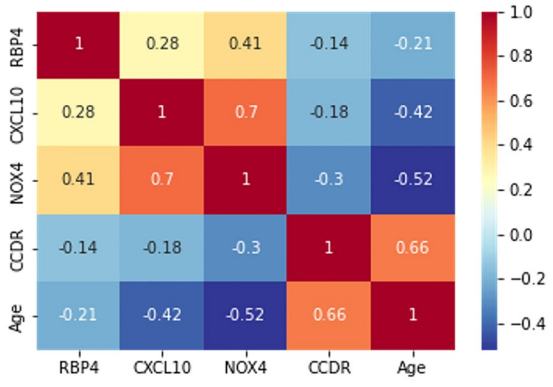

FIGURE 4
The correlation heatmap manifested the correlation between five variables: RBP4, CXCL10, NOX4, CCDR, and age. The color of each cell indicates a high correlation in red and a low correlation in blue, with the darker color indicating the strength of the correlation.

TABLE 3 Machine learning results summary for the normal and MCI.

Feature	Algorithm	AUC	ACC	Sensitivity	Specificity	Precision	F1
RBP4, CXCL10	Random Forest	0.77	0.76	0.77	0.77	0.78	0.77
RBP4, NOX4	SVM[1]	0.84	0.83	0.89	0.79	0.82	0.84
CXCL10, NOX4	Extra Tree	0.78	0.78	0.83	0.74	0.74	0.77
RBP4, CXCL10, NOX4	SVM[1]	0.64	0.66	0.91	0.38	0.61	0.73

[1] Support vector machine.

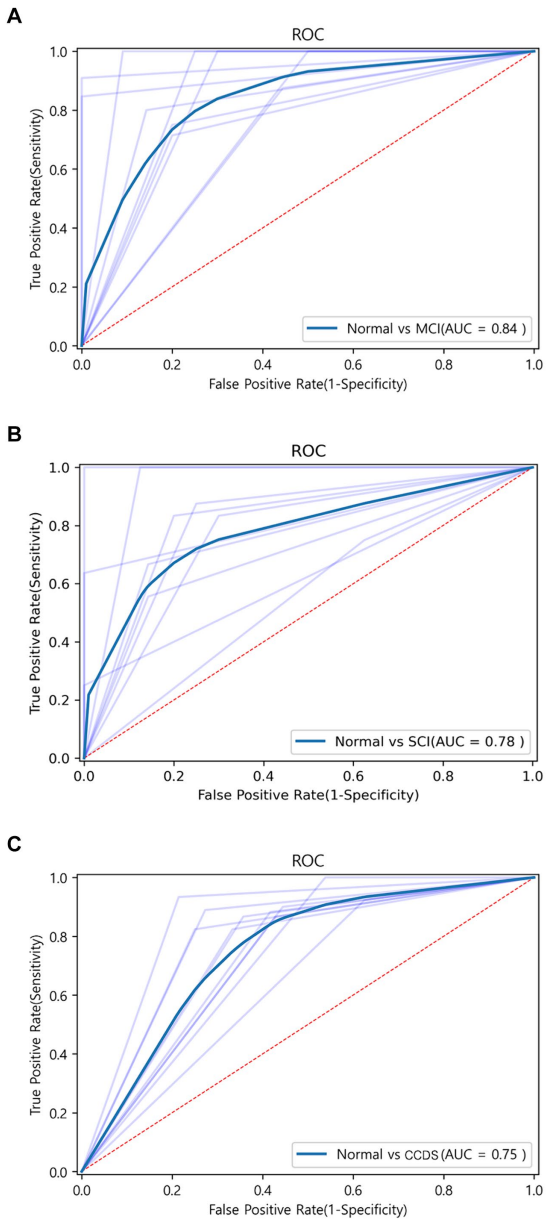

FIGURE 5
ROC curve graph between each group obtained through machine learning. **(A)** ROC curve for the combination of RBP4 and NOX4 between normal and MCI using the SVM algorithm. AUC is shown as 0.83. **(B)** ROC curve for the combination of RBP4, CXCL10, and NOX4 between normal and SCI using the Extra Tree algorithm. AUC is shown as 0.78. **(C)** ROC curve for the combination of RBP4 and NOX4 between normal and CCDS using the SVM algorithm. AUC is shown as 0.75.

analysis result among the seven algorithms (F1 score of 0.75) (Table 4). Despite the small sample size, the use of a combination of the three genes proved to be the most effective set for distinguishing between healthy and SCI patients. Utilization of the ROC curve for predicting favorable outcomes in the normal and SCI states was based on animal plasma analysis (Figure 5B).

3.3.4 Normal and CCDS state prediction

For the discrimination between normal and CCDS, the combination of RBP4 and NOX4 biomarkers showed the highest performance with an F1 score of 0.81. In contrast, the combination of CXCL10 and NOX4 exhibited the lowest performance, with an F1 score of 0.74 (Table 5). When utilizing all three genes, resulted in an F1 score of 0.79 (Table 5). The comparison analysis between normal and CCDS samples demonstrated that the combination of RBP4 and NOX4 is the most effective set for distinguishing between the two states. The utilization of ROC curves for predicting favorable results in normal and CCDS conditions was based on plasma analysis (Figure 5C).

4 Discussion

Despite notable progress in veterinary technology and the increased lifespan of CAs owing to heightened awareness among owners (43), advances in diagnostic technologies for unpredictable cognitive disorders still need to be made. The increasing number of geriatric animals has led to an increase in medical expenses which poses a considerable financial and psychological burden when diagnosing and managing cognitive disorders, such as CCDS, is delayed or missed (44). The difficulty of definitively determining cognitive decline in older animals through CCDR evaluation complicates the diagnosis of CCDS (45). CA owners may overlook subtle deteriorations in the condition of their animals, especially when overt symptoms of cognitive dysfunction are absent (4). This oversight can delay the diagnosis and treatment of CCDS. While the CCDR system is functional, relying solely on it and the subjective assessments of owners and veterinarians may not provide a comprehensive evaluation of the cognitive function of the animal in CCDS cases (6, 45, 46). Collectively, if objective indicators are introduced to differentiate between healthy animals and those exhibiting cognitive dysfunction based on CCDR scores, veterinarians can collaborate with CA owners to formulate precise treatment strategies with greater diagnostic certainty. A comprehensive list detailing the health statuses of the animals involved in the experiments is provided in Supplementary Table S1.

As we mentioned in "2.1.4. Canine Experimental Group" in "Materials & Methods," the CCDR score used in our study differs

TABLE 4 Machine learning results summary for the normal and SCI.

Feature	Algorithm	AUC	ACC	Sensitivity	Specificity	Precision	F1
RBP4, CXCL10	Bagging	0.59	0.61	0.55	0.64	0.53	0.59
RBP4, NOX4	SVM[1]	0.62	0.64	0.92	0.33	0.63	0.73
CXCL10, NOX4	Bagging	0.70	0.69	0.72	0.67	0.72	0.70
RBP4, CXCL10, NOX4	Extra Tree	0.78	0.77	0.73	0.83	0.82	0.75

[1] Support vector machine.

TABLE 5 Machine learning results summary for the normal and CCDS.

Feature	Algorithm	AUC	ACC	Sensitivity	Specificity	Precision	F1
RBP4, CXCL10	Extra Tree	0.71	0.71	0.75	0.67	0.78	0.75
RBP4, NOX4	SVM[1]	0.75	0.77	0.89	0.61	0.75	0.81
CXCL10, NOX4	XgBoost	0.69	0.69	0.77	0.60	0.74	0.74
RBP4, CXCL10, NOX4	Extra Tree	0.72	0.72	0.77	0.67	0.78	0.79

[1] Support Vector Machine.

somewhat from the score intervals for Normal, MCI, and SCI used in CCDR score (12).

When we initiated this study, veterinarians from the hospitals that provided the CCDR questionnaires used by Salvin et al. (12) indicated that specific behavioral items were challenging for dog owners to respond to, specifically items 7 ("How often does your dog have difficulty finding food dropped on the floor?"), 11 ("Compared with 6 months ago, does your dog have difficulty finding food dropped on the floor"), and 13 ("Compared with 6 months ago, is the amount of time your dog spends active?") (12). The veterinarians expressed concerns that the owners' recollections might not be sufficiently accurate to provide reliable responses for these items, potentially leading to erroneous scores that could adversely affect the machine-learning analysis. Considering that the CCDR score depends on the owners' responses, we deemed it prudent to exclude cases where response accuracy might be compromised to ensure a more reliable machine-learning analysis. Consequently, we decided to exclude the scores for these three items from the total CCDR score. As a result, the scoring ranges for Normal, MCI, and SCI appear different from those proposed by Salvin et al. (12), since we omitted the scores for the three specified items from the total CCDR score. It is important to note that this adjustment does not imply any deficiencies in the study by Salvin et al. (12). Instead, it reflects considerations made by veterinarians in the clinics, tailored to the design and specific requirements of our research.

This study explored novel biomarkers using methodologies similar to those used to identify effective biomarkers of classical AD and PD in experimental animals (Figure 1). Our selection of the potential biomarkers RBP4 and CXCL10 deliberately excluded well-documented biomarkers and those encumbered by intellectual property rights (Supplementary Figures S1, S2). We also incorporated NOX4, anticipated as a notable indicator in various neurological disorders (26, 27, 33, 47). We hypothesized that this approach would enhance the ability to effectively distinguish cognitively impaired animals from their healthy counterparts. The ability to differentiate between normal and cognitively impaired animals via blood analysis is expected to provide a substantial opportunity for making early diagnosis of cognitive disorders and delaying the pathological progression through various therapeutic interventions. However, these endeavors have been markedly constrained by the blood–brain barrier and practical limitations in clinical settings (48, 49). Contrary to our initial hypothesis, the three identified biomarkers demonstrated lower expression levels in the blood of both the experimental animals and those with CCDS than in their healthy counterparts. However, our finding of lower expression levels of these biomarkers in CCDS-afflicted animals was initially unexpected, given their previously documented elevated levels in AD and PD (26, 27). Nonetheless, further analysis confirmed this pattern, suggesting that expression levels may vary selectively based on tissue characteristics. Specifically, in PD models, a marked increase in NOX4 expression is not observed in brain regions other than the hippocampus, highlighting the potential for a differential expression depending on the specific pathology and tissues involved (26). RBP4 belongs to the lipocalin family and serves as the primary transporter for hydrophobic retinol, which is also referred to as vitamin A (29). RBP4 is a plasma protein that specifically binds to retinol and acts as its transporter in the circulation (50). This protein is primarily synthesized in the liver before entering circulation (29). Its influence on the body, shaped by protein expression patterns and interactions with receptors, is complex, leading to various hypotheses (29). Apart from the liver, it is present in the retinal pigment epithelium, testes, adipose tissue, muscle tissue, brain, and choroid plexus (29, 51–53). While the precise function of RBP4 in the central nervous system remains unclear (51, 52), studies have shown that RBP4-deficient mice display decreased mobility and anxiety-like behavior, along with neuronal loss and gliosis in the cerebral cortex and hippocampus (29). Furthermore, evidence suggests a reduction in neuroblast proliferation in the subventricular zone (31). Specifically, the RBP binding site is localized in the endothelium surrounding the choroid plexus, enabling substantial transportation of retinol through the blood–brain barrier (51). The CXC chemokine ligand (CXCL10) is believed to have a significant impact on neuroinflammatory conditions (54), potentially affecting neuronal cells and astrocytes (33). As per Bajova et al., persistent CXCL10 stimulation in the culture model triggers ERK1/2,

CREB, and NF-kB pathways, leading to enhanced levels of anti-apoptotic BCL-2 proteins and antioxidant enzymes like manganese superoxide dismutase (SOD2), which offer protection against superoxide radicals (33). The findings of this study indicate a potential close correlation between the presence of CXCL10 and its neuroprotective effects. While the efficacy and associated mechanisms of the three newly suggested biomarkers for early detection of CCDS require further exploration, there is an expectation that they could be beneficially applied in neurodegenerative conditions in canines.

Securing blood samples from outpatients required obtaining consent from their guardians, which notably protracted the sample collection timeline. Moreover, concerns regarding the integrity of the stored blood samples imposed restrictions on the duration of the collection period for analysis. Despite these challenges, the samples utilized here yielded high accuracy and prediction using machine-learning algorithms, indicating that various combinations of biomarkers could distinctly differentiate between normal and cognitively impaired dogs. This outcome underscores the potential of these biomarkers in advancing the diagnostic capabilities of CCDS. A study sought to differentiate between healthy individuals and patients by training machine-learning algorithms using brain imaging data from human patients with AD. However, this approach primarily aimed to ascertain the presence of AD in patients based on existing imaging results (55). In essence, it was not an endeavor to proactively identify individuals deviating from the normative range at an early stage but rather a retrospective confirmation of AD in already diagnosed individuals. Taking this into account, it is highly encouraging that the predictive capability for CCDS is effectively enhanced through the identification and combination of novel blood biomarkers. In a comparison of normal versus MCI and normal versus CCDS, the pairing of the biomarkers RBP4 and NOX4 yielded optimal outcomes with the application of support vector machine (SVM) methods.

Conversely, to distinguish between normal and SCI states, the most effective results were obtained using the Extra Tree algorithm, which incorporated the full spectrum of biomarkers. The complete results of the SVM analysis for various biomarker combinations are shown in Supplementary Tables S2–S4. This outcome suggests that the potency of the predictive algorithm can be further refined and improved by acquiring additional samples in future studies, thereby advancing the early detection and management of CCDS. In other words, although our machine-learning analysis was conducted with a limited number of samples, the accuracy and predictive capability achieved are promising. It is anticipated that with additional sample data, the errors in these metrics will diminish progressively, enhancing their reliability as objective indicators in clinical practice. These results are expected to serve as valuable standards for guiding treatment decisions. Historically, there has been considerable skepticism regarding the feasibility of detecting early biomarkers of cognitive impairment through blood, a sentiment prevalent in both human and veterinary medicine. In canine studies, although certain biomarkers have been identified in the blood, their lack of discriminatory power has rendered them impractical for clinical application (3, 4, 18, 20, 21, 56, 57). We are dedicated to identifying viable biomarkers of dementia-related cognitive impairment and to building a substantial understanding of the pathological mechanisms of these biomarkers. Therefore, we did not rely solely on comparing the ELISA measurements of suspected cognitive impairment biomarkers. Instead, we employed advanced machine-learning analysis tools to assess the existence of CCDS for each unique combination of identified biomarkers, thereby enhancing the precision and applicability of our findings in clinical settings. In other words, if we were simply trying to differentiate between MCI and SCI based on the level of a single biomarker, there would be no need to apply machine learning techniques to train on the combination of results from multiple biomarkers. It is important to remember that a high CCDR score does not necessarily imply a proportional decrease in biomarker expression levels in the blood. Thus, we utilize machine learning with multiple biomarkers as variables because it is challenging to distinguish between Normal, MCI, and SCI using a single biomarker.

Our discoveries show great promise as a means of evaluating the cognitive health of elderly animals in clinical environments, potentially improving the well-being of companion animals and their caregivers. Regular monitoring of these biomarkers could act as a valuable indicator for identifying cognitive impairment early on, thereby enabling early diagnosis and intervention. It's worth noting that in our study, elderly animals were categorized into MCI and SCI groups based on CCDR scores provided by caregivers, rather than by veterinarians' long-term observations. The assignment of scores in the CCDR could be influenced by unrelated conditions, such as cataracts leading to blindness. This indicates a bidirectional association between sensory impairment and behavioral changes, potentially indicative of CCDS (58). While our proposed new biomarker combination might not exhibit a distinct statistical variance between MCI and SCI, it's important to recognize the subjective nature of the CCDR score itself. Our goal is to introduce innovative biomarkers that can detect early signs of cognitive decline and offer insights into disease progression. Therefore, gathering additional clinical samples is essential. Moreover, by observing how these biomarkers respond to treatment in animals diagnosed with CCDS, we aim to assess the effectiveness of our biomarkers. This endeavor mirrors our aspiration to expand our dataset and refine our methodology for identifying cognitive impairment in companion animals, while also differentiating between different forms of degenerative brain disorders. This advancement may pave the way for tailoring treatment strategies, allowing veterinarians to provide precise and prompt care to geriatric animals in clinical settings. This advancement will potentially enable the development of more nuanced treatment strategies, empowering veterinarians in clinical practice to provide targeted and timely care to aging animals. Several dogs classified as MCI or SCI were reported not to have been diagnosed with CCDS but instead exhibited neurological conditions such as MUO (including GME and NME), meningitis, and hydrocephalus in Supplementary Table S1. When looking at the data, these disorders may present with forebrain signs, including restlessness and behavioral changes, which are likely to result in higher scores on the CCDR, a scoring system that relies on clinical observations. These disorders can manifest as forebrain signs, including restlessness and behavioral changes, which are likely to result in higher scores on the CCDR—a scoring system that relies on clinical observations. Another point to consider is whether dogs with a history of brain diseases are more predisposed to developing CCDS later in life, a phenomenon already reported about idiopathic epilepsy in dogs (59, 60). Therefore, in the future, more clinical case data and additional biomarkers will be needed to improve the ability to distinguish between other brain diseases and cognitive impairment.

5 Conclusion

We are dedicated to identifying biomarkers that can be used to diagnose cognitive impairment early in elderly animals, thereby providing a critical window for appropriate therapeutic intervention. The source of clinical samples is of paramount importance in this quest as the blood–brain barrier poses substantial challenges in detecting prodromal symptoms of cognitive impairment in both humans and animals. Despite these obstacles, we posit that with the aid of this diagnostic tool, companion animals can enjoy prolonged quality of life alongside their owners, who, in turn, may experience reduced psychological distress as a result of mitigating the behavioral changes associated with severe cognitive decline in their pets. We advocate early screening for cognitive impairment through blood tests, leveraging the predictive values derived from CCDR scores. This approach empowers veterinarians to diagnose and initiate treatment strategies for animals that are likely to develop CCDS and to monitor their brain health through regular follow-up testing. The predictive accuracy and sophistication of our model are expected to improve as we refine our methodology and expand our sample size. Given the substantial diagnostic value demonstrated thus far, we are optimistic regarding the clinical applicability of our method. Looking ahead, we envision that the early detection capabilities of companion animals will pave the way for similar advances in human medicine, thereby broadening the scope and impact of our research.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary material.

Ethics statement

The animal studies were approved by the Institutional Animal Care and Use Committee (IACUC) of Soonchunhyang University. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent was obtained from the owners for the participation of their animals in this study.

Author contributions

CKi: Methodology, Validation, Writing – original draft. JK: Methodology, Software, Writing – original draft. SYo: Methodology, Software, Writing – original draft. IY: Investigation, Methodology, Writing – original draft. HL: Methodology, Writing – original draft. SS: Methodology, Writing – original draft. DK: Methodology, Writing – original draft. SoK: Methodology, Writing – original draft. S-AK: Conceptualization, Methodology, Writing – review & editing. CKw: Methodology, Writing – original draft. SYi: Conceptualization, Data curation, Funding acquisition, Supervision, Writing – original draft, Writing – review & editing.

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Conflict of interest

JK and SY were employed by the iCONNECTOME, Co., Ltd., HL was employed by the iamdt, Co., Ltd., and SK was employed by the GenesisEgo, Co., Ltd.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2024.1390296/full#supplementary-material

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Of potential new treatment targets and polythetic approach in meningoencephalitis of unknown origin: a review

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Meningoencephalitis of unknown origin (MUO) represents an umbrella term for inflammatory, non-infectious central nervous system (CNS) diseases in dogs. Current therapeutic approaches, involving long-term glucocorticosteroid use, often fail to provide adequate relief or cure, and the effectiveness of additional immunosuppressive medications remains uncertain. Future advancements in MUO treatment may benefit from patient-specific therapies, potentially enhancing treatment precision, efficacy, and minimizing side effects. However, significant challenges impede this progress, including ambiguity in MUO subtype classification, uncertainties regarding the autoimmune nature vs. infectious triggers, and the lack of reliable diagnostic biomarkers. Clinical heterogeneity and overlapping signs with other encephalopathies further complicate diagnosis and treatment. This review gives an overview about diagnostic findings and immunological features of MUO. It advocates for a more overall characterization of MUO by using a polythetic system to better characterize MUO subtypes, identify immunological treatment targets, and establish a conceptual foundation for future therapeutic trials. Addressing these themes may lead to more effective and less burdensome treatments, improving the quality of life for dogs afflicted with MUO and their owners.

KEYWORDS

canine (dog), meningoencephalitis of unknown origin (MUO), diagnostic, clinical signs, immunology

1 Introduction

Meningoencephalitis of unknown origin (MUO) is an inflammatory, non-infectious disease of the central nervous system (CNS) in dogs (1, 2). MUO primarily functions as an umbrella term, frequently utilized for diagnostic purposes in the absence of histopathological confirmation and specific classification (3, 4). Most commonly mentioned subtypes encompass granulomatous meningoencephalomyelitis (GME), necrotizing meningoencephalitis (NME), and necrotizing leukoencephalitis (NLE) (4) often summarized as necrotizing encephalitis (NE) (2). Less common entities, such as eosinophilic meningoencephalitis, greyhound encephalitis, idiopathic cerebellitis, and autoantibody encephalitis are occasionally excluded from the MUO category in certain literature (4). Steroid-responsive meningitis-arteritis (SRMA), a predominant neutrophilic meningitis and vasculitis, remains a distinct entity and is not subsumed within the MUO terminology (4, 5).

MUO lacks an identifiable infectious trigger and typically respond positively to immunosuppressive therapy, primarily through long-term glucocorticosteroid application

(4, 6–9). Despite a variable and sometimes insufficient response to therapy and the high incidence of side effects, the treatment regimen has remained largely unchanged for the last 10 to 20 years (9).

The current state of therapeutic approaches for MUO in cats and dogs is unsatisfactory. Long-term therapy with glucocorticosteroids improves the outcome in treated patients compared to untreated patients; but the efficacy of commonly used additional immunosuppressive medication remains inconclusive (9–11). This treatment results in often intolerable burden of side effects and failing to sufficiently provide long-term relief from clinical manifestations, let alone cure the disease.

For the future, a patient-specific therapy would be desirable as it might have several advantages (12):

Precision medicine: Patient-specific therapy might allow for a more precise and targeted approach to treatment. By understanding the unique characteristics of the pet's autoimmune CNS disease, medications and interventions could be customized to address the specific mechanisms driving the condition. Tailoring treatment to the individual patient's needs could enhance the effectiveness of interventions while minimizing potential side effects.

Optimized efficacy: Different dogs may respond differently to standard treatments. Personalized therapy takes into account the specific molecular and cellular factors contributing to the autoimmune response and the stage of the disease, increasing the likelihood of a more effective treatment outcome.

Minimized side effects: Standard treatments for autoimmune diseases often involve pan immunosuppression that can affect various bodily functions (13). Tailoring therapy to the individual patient might help minimize unnecessary exposure to medications, reducing the risk of adverse effects.

Improved quality of life (QoL): By customizing treatment to the unique characteristics of the dog's autoimmune CNS disease, veterinarians could optimize the balance between suppressing clinical signs and preserving the dog's overall wellbeing. This can lead to an improved quality of life for the patients and their owners.

Development of satisfactory and patient-specific treatment necessitates a profound understanding of the etiopathogenesis of MUO, identifying potential treatment targets. Three significant challenges hinder this understanding:

First, the ambiguity persists regarding whether all MUO subtypes constitute a single disease entity or if histopathological subtypes represent distinct independent diseases with varying etiopathogenesis (2).

Second, uncertainties remain regarding the clear autoimmune nature of these diseases or the potential involvement of a relevant infection triggering and initiating excessive inflammation (9).

Third, there is a lack of consensus on how to diagnose MUO or its subtypes ante mortem without histopathological confirmation via biopsy, which is an expensive, time-consuming, and potentially risky procedure, not routinely employed. Currently, no single biomarker or combination thereof reliably establishes the diagnosis of MUO or distinguishes between subtypes (14). Clinical signs and laboratory findings of dogs with MUO exhibit heterogeneity and occasionally, MRI and/or cerebrospinal fluid (CSF) findings are normal: Abnormal MRI with normal CSF is present in 9%, normal MRI and abnormal CSF in 6%, and normal MRI and CSF in 3% of dogs with MUO (15), in general 13% of dogs with MUO show normal CSF (4). Dogs of any breed and age may be affected, challenging breed specific predisposition patterns. Clinical signs and findings also overlap significantly with other encephalopathies (16). Strict adherence to previously suggested definitions of MUO would exclude a considerable number of affected dogs and could even include dogs with other diseases (4).

To advance new and more effective therapies with less side effects for patients with MUO, this review aims to lay the conceptual foundation for future therapeutic trials by addressing the following overarching themes:

- Characterizing diverse MUO subtypes beyond the conventional subtypes of GME and NE observed in dogs.
- Identifying potential treatment targets within the immunological disease process.
- Advocate for a more overall characterization of MUO by using a polythetic approach.

2 History of MUO

First described in 1968, GME was initially referred to as reticulosis and predominantly considered neoplastic (17). However, in the subsequent decades, particularly in the 70s and 80s, it became increasingly evident that this entity is more appropriately characterized as an inflammatory disease (18–23). Despite extensive investigations, no infectious agents were identified, and the underlying cause remained unclear (18, 19). Various theories were postulated, including the suggestion that GME might represent an aberrant reaction to canine distemper virus infection or vaccination, a retrovirus infection stemming from vaccine contamination, or involvement of other viral, parasitic, or infectious agents (24). None of these theories could be substantiated through immunohistochemical examinations, polymerase chain reaction (PCR) analyses, viral inoculation, or microbiological cultures, failing to reveal a conclusive infectious agent triggering the inflammatory response (8, 24–26). Early reports also proposed an overstimulation of the immune system due to the antiparasitic drug levamisole (27) or a T-cell-mediated delayed-type hypersensitivity reaction as potential causes for GME (24, 28); however, none of these hypotheses could be definitively proven.

Subsequently, NME- in Pug dogs often referred to as Pug dog encephalitis due to its strong breed disposition (29)- and NLE in Yorkshire Terriers were documented (6). Once again, no infectious agents were identified (25, 30).

Clinical examination, CSF analyses, and computed tomography (CT) scans proved insufficient in differentiating between the various histopathological subgroups (4, 31). As a result, these meningoencephalitides were amalgamated for clinical purposes and described under diverse names such as idiopathic inflammatory brain disease, non-infectious meningoencephalitis, and sterile meningoencephalitis, among others (1, 2, 4, 32). Over time, MUO has emerged as the prevailing and widely accepted

term (4, 33). Presently, MUO stands as one of the most common encephalitis, accounting for 52%−69% of dogs with encephalitis (5, 34).

Despite the array of names and classifications, therapeutic approaches have shown limited evolution in recent years (4, 9). Early manuscripts outlined the utilization of glucocorticosteroid therapy in individual dogs diagnosed with MUO (19). Given the variability in outcomes, additional immunosuppressive drugs such as mycophenolate mofetil, leflunomide, cyclosporine, azathioprine, and even radiation protocols were introduced alongside glucocorticoids (35–44). While the available data may exhibit some contradictions, on the whole, none of these therapies seems to demonstrate superiority over others in terms of efficacy and side effect profiles (9). Furthermore, uncertainties persist regarding whether combination therapy offers a significant advantage over the use of glucocorticoids alone (11, 45, 46).

To the author's knowledge, there are no studies evaluating the effectiveness or side effect profiles of other immunosuppressive drugs without concurrent application of glucocorticosteroids in MUO (9). Additionally, there is only one comparative study assessing combined therapy with glucocorticosteroids and an additional immunosuppressive drug against treatment with glucocorticosteroids alone in a prospective, double-blinded approach (9, 11).

Presently, one of the most commonly employed treatment regimens entails the administration of parenteral cytarabine in conjunction with long-term glucocorticosteroids (10). However, the prolonged use of glucocorticosteroids is associated with numerous side effects, including polyuria/polydipsia, gastrointestinal symptoms, alopecia, calcinosis cutis, and others (13, 47). These side effects significantly impact QoL of the dog and the owner (48). Attempts to reduce glucocorticoid doses often result in deterioration or relapse of clinical signs (40). Even with appropriate doses, treatment frequently yields insufficient clinical improvement, and the mortality rate of MUO can reach up to 30%−56% within 100 days after diagnosis (33, 40, 49).

For a detailed review on therapeutic options used at the moment and treatment results, we would like to refer the reader to the latest reviews, for example by Jeffery and Granger (9).

3 Clinical diagnosis of MUO

Clinical signs vary with lesion localization and reflect focal or multifocal lesions in the CNS, commonly involving seizures, ataxia, proprioceptive deficits, vestibular signs, blindness, and multiple cranial nerve deficits, sometimes accompanied by head or neck pain (24). Systemic signs like fever are rare (4). GME mainly causes signs of lesions in the forebrain, brainstem, or both (4, 19) and blindness in the ocular variant (50, 51). Large breed dogs presented significantly more often with decreased mentation compared to small breed dogs (33). NE mainly causes signs of forebrain lesions with seizures (6, 29, 30, 52, 53). Rarely, GME can occur as meningomyelitis and cause paresis and spinal ataxia, with approximately 9.5%−13% involving the spinal cord only (4, 54, 55).

To assess the severity of clinical signs objectively, two neurodisability scales (NDS) have been developed so far (37, 56) which attribute scores to different clinical signs. While Goncalves et al. (5) showed good interobserver agreement in prospective cases, this was worse in retrospective evaluation of patient records. Therefore the authors do not encourage the use of this score for retrospective data (56). Which of the scores is superior at any level was not evaluated so far, but it seems they show good correlation (57).

Pug dogs with NME tend to be younger (median 18 months) than other dogs with NE (2–4 years), and dogs with GME tend to be older (median 55 months; range: 6 to 144 months) when the first signs of encephalopathy occur (2, 4, 53).

Diagnostics may involve advanced imaging, preferably magnetic resonance imaging (MRI), of the brain and/or spinal cord, CSF analysis, and exclusion of regional infectious agents (4).

MRI findings can vary significantly, but the classic presentation typically consists of multifocal, intra-axial, ill-defined lesions with mild mass effect and inhomogeneous contrast enhancement (33). But MRI findings in MUO can be normal in up to 7%−9% (4, 15).

In GME, MRI reveals focal, multifocal, or diffuse T2-weighted (T2w) and fluid attenuating inversion recovery (FLAIR) hyperintense lesions in the forebrain, brainstem, or cerebellum in both white and gray matter. The degree of contrast enhancement in the CNS parenchyma varies, with little to minimal contrast enhancement of the meninges (58).

MRI findings for NE differ slightly among affected breeds but exhibit significant overlap. In Yorkshire Terriers with NE, lesions are mostly multifocal, uni- or bilaterally asymmetrical lesions in the forebrain, including the diencephalon. Predominantly, these lesions occur in the periventricular and subcortical white matter, often sparing the cortical gray matter (59, 60). The brainstem is often less severely affected, while the cerebellum and spinal cord typically remain unaffected. These lesions are usually T2w and FLAIR hyperintense, although in more chronic cases, FLAIR signal might be suppressed (59, 60). Contrast enhancement is mostly mild and inhomogeneous to patchy (59).

MRI in NE in Pug dogs and Chihuahuas exhibit multifocal or diffuse, asymmetrical forebrain lesions, most severe in occipital and parietal lobes, with the frontal lobes less frequently affected. Diencephalic lesions are less common, and brainstem or cerebellar lesions are possible but rare (61). The border between white and gray matter is often blurred, and most lesions are present in the gray matter. Lesions are usually T2w and FLAIR hyperintense, although in more chronic lesions, FLAIR signal might be suppressed (59, 60). Contrast enhancement is mostly mild and inhomogeneous to patchy (59).

CSF shows increased protein and predominantly mononuclear pleocytosis, but mixed to neutrophilic pleocytosis, albuminocytologic dissociation are not uncommon, and up to 22% of dogs with MUO display normal CSF (4). C-reactive protein (CRP) in CSF is not significantly different from healthy dogs (62).

Blood tests and extracranial findings are generally within physiological limits, which is often in contrast with infectious meningoencephalitis. For instance, in cases of neosporosis, blood creatine kinase activity may be elevated (63).

Based on clinical signs, blood tests, MRI, and cerebrospinal fluid findings, as well as the patient's place of residence and

travel history, possible infectious agents should be excluded with appropriate investigations. There can be no uniform recommendation for all cases, as the appropriate pathogen investigations can vary significantly depending on the individual case.

For research purposes, currently, the most widely adopted inclusion criteria to diagnose MUO encompass the following points (4):

- Age: > 6 months.
- Evidence of a multifocal CNS disease demonstrated by either multifocal or diffuse lesions suspected after the neurological examination and multiple, single, or diffuse intra-axial hyperintense lesions on T2w MR images, or a unifocal lesion suspected after the neurological examination and multiple or diffuse intra-axial hyperintense lesions on T2w MR images.
- CSF analysis should be hypercellular, with >50% mononuclear cells (preferably monocytes/lymphocytes).
- Infectious diseases should be ruled out.

The accuracy of these inclusion criteria has not been thoroughly examined and there is debate among researchers on these points. Recent reports about MUO in dogs younger than 6 months might additionally question the age at inclusion (64).

Diagnosis can be complicated by overlapping clinical, MRI, and CSF findings with other conditions such as neoplasia (16). Confirming the diagnosis requires histopathological confirmation of sterile inflammation (65). Therefore, the identification of the histopathological subtype of GME or NE through clinical diagnosis alone is limited. Due to this limitation, a uniform definitive consensus on the diagnosis of MUO for clinical or research purposes has not been established (14).

4 Prognostic factors of MUO

Prognosis is guarded with MUO, and various diagnostic indicators appear to be linked to mortality risks (2, 3, 33, 35, 40, 46, 55, 66, 67). Clinical manifestations such as reduced mentation, seizures, and signs related to multifocal or caudal cranial fossa lesions are indicative of a less favorable prognosis (55). In general, severity of clinical signs expressed as higher clinical NDS seem to be associated with 1-week-survival but not with long term survival in dogs with NE (56, 57).

Additionally, dogs with higher body weight or advanced age and juvenile dogs tend to face a worse outcome (33, 64, 67).

Elevated and/or neutrophilic CSF cell counts, and hyperlactatemia are correlated with shorter survival times (66, 67).

MRI lesions seem to be correlated with prognosis to some degree. Severe MRI findings, such as the loss of the CSF signal of the cerebral sulci and foramen magnum herniation, are associated with an increased risk of mortality (40). High T2w lesion burden might be correlated with worse long term prognosis (68) while unremarkable MRI findings seem to be associated with better prognosis (69). Increased contrast enhancement might be correlated with increased risk of relapse (68).

5 MUO in cats

MUO is not limited to dogs, with infrequent reports of its occurrence also in cats (1, 70). Additionally, although not explicitly named "MUO," feline meningoencephalitides have been documented from authors proposing an infectious agent without having been able to identify it (71–73). A similar scenario is observed in Staggering disease: The term Staggering disease describes a clinical syndrome caused by non-suppurative, lymphohistiocytic meningoencephalomyelitis (74, 75). Initially believed to result from a viral infection, Borna virus (BoDV-1) was evaluated as a potential causative agent (76). While experimental infections demonstrated BoDV-1-induced neurologic disease in domestic cats (77), consistent detection with independent diagnostic methods proved elusive (78, 79). Consequently, BoDV-1 is no longer considered the causative agent for staggering disease, leaving it classified as a meningoencephalitis with an unknown trigger for some time. Recent evidence, however, suggests that some cases of staggering disease may be caused by Rustrela virus (RusV), as it has been detected in several affected cats (80). Nonetheless, there are still cats with clinical signs of MUO or staggering disease where no infectious agent, including RusV, can be identified (80, 81). This suggests that staggering disease may represent a spectrum, with both unknown (MUO) and infectious causes, depending on the individual case.

In cats, reports on histopathologically confirmed MUO in combination with clinical signs are rare (81). The median age of cats is 7 years, older than the median age of cats typically presented to the clinic for infectious encephalitis (81). The breed distribution of cats with MUO is comparable to the general clinical population, while pedigree cats appeared to be more common among cats with infectious encephalitis (81). Besides acute or chronic neurological signs, systemic signs of illness or blood leukocytosis were frequently present. CSF changes appeared subtle, with albuminocytologic dissociation being the most common finding. Histopathology revealed a multifocal, lympho-histiocytic inflammation in the CNS (81).

Diagnostic and therapeutic strategies for MUO in cats primarily arise from canine MUO research; treatment commonly involves the use of prednisolone (70). However, therapeutic guidance remains largely anecdotal.

6 The search for infectious agents

In canine MUO, several attempts failed to reveal any underlying infectious diseases (8, 24–26, 82). Although some potential infectious agents like bacteria or viruses were identified in individual animals, none were consistently detected across the entire patient cohort, ruling out that they are the underlying cause of MUO (8, 24, 26, 82). This strengthens the hypothesis that MUO might not be triggered by a specific infectious agent but is a genetic disease. On the other hand, RusV was detected in the CNS of cats initially diagnosed with MUO (80). RusV ribonucleic acid (RNA) and antigen were shown by metagenomic sequencing, real-time quantitative polymerase chain reaction (PCR), *in-situ* hybridization, and immunohistochemistry in brain tissues of 27 out of 29 cats with non-suppurative meningoencephalomyelitis

without a previously identifiable cause (80). Screening of possible reservoir hosts in Sweden revealed RusV infection in Wood mice (*Apodemus sylvaticus*) (80). RusV is a relative of the rubella virus and associated with encephalitis in various mammalian hosts, including Wood mice, lions, and wallabies (83–85). It demonstrates a broad host spectrum and extensive geographic distribution, raising the possibility of its involvement in neuropathologies across diverse mammalian species, potentially even humans (80, 83–86).

However, the absence of RusV in some cats with lymphohistiocytic meningoencephalitis shows that MUO might still be a distinct entity in felines, albeit seemingly less prevalent than in canines (80). The divergence in results between cats and dogs with MUO suggests difference in the underlying pathogenic or genetic mechanisms between the two species. While in MUO in dogs a lack of identifiable infectious agents and breed predispositions (4, 82) point toward autoimmune etiology, the prevalence of RusV in cats implies a more prominent role of infectious agents in feline CNS inflammation.

The interspecies difference is most probably influenced by various factors. Lifestyle distinctions, such as dogs being more leash-restricted and less prone to consuming prey, may reduce their exposure to infectious agents (87). In contrast, the outdoor habits of cats, including hunting, may increase their vulnerability to pathogens like RusV.

The broader exposure to antigens during their outdoor pursuits could contribute to the development of a more diverse and potentially regulated immune system in cats, potentially reducing the susceptibility to autoimmune disorders: The so called "hygiene hypothesis" proposes that reduced early-life exposure to infections and a cleaner, more sanitized environment may contribute to the increased prevalence of autoimmune diseases (88). This theory suggests that limited microbial exposure early in life may lead to an improperly regulated immune system, increasing the risk of allergic and autoimmune diseases (88, 89). Additionally, gastrointestinal parasite infections are immune modulatory (90), and the potential predisposition of cats to such infections due to their outdoor lifestyle might save them from overreacting immune responses, although, no discernible difference in deworming practices between cats and dogs emerges from existing studies (87, 91).

In human medicine, there is a latitude difference in prevalence of Multiple sclerosis (MS): Individuals residing in countries closer to the equator during their initial years of life exhibit a lower incidence of MS possibly related to sunlight exposure and higher vitamin D levels (92, 93). Pets like dogs have a different mechanism of synthesizing vitamin D (94, 95), but a certain influence from exposure to sunlight might still be possible. The outdoor lifestyle of cats may influence the developing immune system differently compared to the immune system of young dogs mostly held indoors. It might be possible that early exposure to sunlight is beneficial to developing a healthy immune system (89).

Most likely, the cause for an increased prevalence of autoimmune CNS disease in dogs compared to cats is the difference in the genetic background (6, 29, 96–98). Cats, especially the more common European shorthaired cats, may be less inbred than dogs, leading to a reduced genetic predisposition to MUO.

7 Genetic base of MUO

A genetic basis for MUO is highly probable, and distinct breed-specific patterns are evident (6, 52, 55, 59). NE predominantly affects toy breeds, while NME is prevalent in breeds such as Pug dogs, Maltese, or Chihuahuas (29, 52, 99). On the other hand, NLE is more commonly observed in Yorkshire Terriers and French Bulldogs (6, 30, 59). The distribution of GME appears more heterogeneous, primarily affecting toy and terrier breeds, but approximately one third of affected dogs belong to larger breeds with a body weight exceeding 15 to 20 kg (4, 33, 55, 100).

In the context of genetic predisposition, heritability of NME specific to the Pug dog is 0.67 (101). NME is particularly associated with the Major Histocompatibility Complex II (MHC II) haplotype featuring DRB1-010011, DQA1-00201, and DQB1-01501 (102, 103). Also, Maltese dogs and Chihuahuas seem to be at increased risk to develop MUO with a certain MHC II haplotype (99, 104). MHC II plays a crucial role in antigen presentation and has been correlated with various autoimmune diseases in both canines and humans, including Vizsla polymyositis and MS (105, 106).

8 Histopathological findings in MUO and classification of MUO subtypes

MUO mostly serves as an umbrella term, predominantly employed for diagnostic purposes in the absence of histopathological confirmation and classification. Noteworthy subtypes include GME and necrotizing encephalitis (NE) (comprising NME and NLE). Less frequent entities such as eosinophilic meningoencephalitis, greyhound encephalitis, optic neuritis, idiopathic cerebellitis, and other unclassified sterile meningoencephalitides are occasionally excluded from the MUO category in some publications (4).

Histopathological characteristics exhibit specificity for each MUO subtype. GME is classified by asymmetric angiocentric or nodular granulomatous lesions arising from the focal eccentric nodular proliferation of macrophages within histiocytic perivascular cuffs in the Virchow-Robin space, primarily evident in the cerebellum, medulla oblongata, and spinal cord (19–21, 107). NE manifests as non-suppurative perivascular inflammation and necrotic lesions predominantly in the white matter of the cerebrum and brain stem, or the gray matter and meninges of the telencephalon in NLE or NME respectively (6, 29, 30, 107).

GME, NME, and NLE all show a predominance of CD3-positive T cells, along with macrophages and plasma cells. The differences between these MUO subtypes are relatively subtle (108). In NME and NLE, macrophages are frequently observed in the malacic neuroparenchyma, where they likely help remove cellular debris (108). In contrast, in GME, macrophages are more commonly found in the perivascular cuffs, suggesting their role in forming granulomatous lesions as part of the immune response (108).

In NME and NLE, CD3-positive T cells adhere to astrocytes in malacic regions, with this interaction occurring in different areas: in the cortex for NME, and in the white matter for NLE and GME (108). Furthermore, astrocytes stain positive for IgG in

NME and NLE (but not in GME), in distinct regions—protoplasmic astrocytes in the cortex in NME and fibrous astrocytes in the white matter in NLE (108, 109). This suggests that different target structures may be involved in the inflammatory processes of NME and NLE.

However, the distinctiveness of histopathological features is not universally observed, with frequent overlap between NME and NLE, leading to their collective designation as NE (2).

Although there is general scientific agreement that typical and distinct features regarding age of onset, clinical signs, and histopathologic findings in GME and NE exist, it could be shown that there is more overlap between MUO subtypes and more distinct subtypes than previously known.

In one study, we have shown that it is possible to detect concomitant histopathological features of GME and NE in the brain of a single dog: Microscopically, in four dogs, areas of marked necrosis were evident in the cerebral hemispheres, cerebellar white matter, or brain stem with mainly lymphocytic perivascular infiltrates (110). At the same time, all four dogs also had focal or multifocal high-grade angiocentric granulomatous inflammatory lesions in the cerebrum, and rhombencephalon. Meningitis was found in all dogs. Infectious agents were excluded. This study suggests that there might be additionally significant overlaps between GME and NE. Those dogs were dogs from breeds traditionally considered to suffer from NE variants (6, 30, 52, 99, 110).

Additionally, another breed predisposition in Australian Shepherds was described, which experience MUO at a senior age and likely suffer from GME (100). This underscores an age-dependent susceptibility to MUO in Australian Shepherds.

Furthermore, we have discovered an as-yet-undescribed variant of lympho-histiocytic meningoencephalitis with CNS vasculitis of unknown origin (111). Dogs exhibited clinical signs of severe forebrain disease, rapidly progressing to involve the brainstem, ultimately leading to death. Extracranial clinical signs were only mild (111). MRI examination revealed generalized swelling of cerebral gray matter and subsequent features of increased intracranial pressure, as well as signs of cerebellar and brainstem hemorrhage or transtentorial herniation (111). CSF analysis indicated hemorrhage and lymphocytic dominance in cell differentiation. In necropsy, the brains displayed varying degrees of edema, cerebellar herniation, and hemorrhages. Microscopically, the primary findings comprised lympho-histiocytic inflammation in the brain and/or spinal cord with associated vasculitis (111). An infectious causative agent could not be determined. This highlights that MUO exhibits a much more extensive diversity than previously reported.

This raises the question, what factors contribute to the expression of different inflammatory patterns. The current consensus suggests a multifactorial pathogenesis for MUO (9). Some authors propose a genetic predisposition and a triggering factor like an infectious agent or that exogenous antigens activate T-cells cross-reacting with self-antigens, called molecular mimicry (1, 8, 112). However, as no exogenous triggers have been found in the last 60 years in canine MUO (1, 8, 25, 82, 113), it seems more and more likely that there might be none.

Another theory postulates a multistep pathogenesis of autoimmune disease (114). An autoimmune disease might be caused by a failure of immunological self-tolerance caused by multiple inherited and somatic mutations within the immune system (114). According to current knowledge, autoimmune diseases arise when T and B-cells responding to self-antigens cause misguided and over-reactive inflammation (115, 116).

Physiologically, immunological self-tolerance involves multiple control systems to prevent the accumulation of autoimmune lymphocytes. The first step is central immune tolerance, involving the purging of autoimmune cells in the thymus (117, 118). Here, up to 40% of autoreactive cells escape central immune tolerance (117).

Several subsequent mechanisms are involved in the peripheral immune tolerance to limit auto-reactive immune cell responses (114, 116). Peripheral immune tolerance is enforced through cell-intrinsic (inhibitory pathways) and cell-extrinsic (regulatory T-cells = Tregs) mechanisms (118). Tregs, characterized by their anti-inflammatory properties, suppress autoimmune reactions through various means, including the secretion of anti-inflammatory cytokines (IL-10, TGF-β, and IL-35) and induction of apoptosis in effector cells (119, 120).

Intrinsic regulatory mechanisms involve rendering T-cells non-responsive to antigens (anergy), if they engage a MHC molecule on an antigen-presenting cell without concurrent engagement of co-stimulatory molecules (121, 122). Co-stimulatory molecules, upregulated by pro-inflammatory cytokines during acute inflammation, are essential for T-cell activation. An absence of pro-inflammatory cytokines results in the non-expression of co-stimulatory molecules, leading to anergy (122–125). Therefore, auto-reactive T-cells stay inactive although they have contact with "their" auto-antigen as long as no pro-inflammatory reaction is present.

Moreover, anatomical barriers, such as the blood-brain barrier surrounding CNS parenchyma, can impede the interaction between auto-reactive lymphocytes and antigens (126, 127).

The development of autoimmune diseases necessitates the bypassing of several of these regulatory mechanisms. While a singular gene defect has not yet been identified as the causative factor for MUO, the prevailing hypothesis leans toward multigenetic defects (96, 98, 99, 104, 114). This suggests that the failure of multiple safeguard mechanisms contributes to the development of autoimmune diseases. In the context of genetic predisposition, Pugs exhibit a recognized susceptibility, particularly associated with a specific MHC II haplotype (102). Before even presenting any clinical signs of MUO, asymptomatic pugs already display variations in their immune system and in their serum anti-glial fibrillary acidic protein (GFAP) antibodies can be detected (128, 129). GFAP is mainly part of intermediate filaments in the cytoplasm of astrocytes (130). The presence of anti-GFAP antibodies in the periphery means auto-reactive B cells were activated to produce immunoglobulins (131). These findings indicate that antigen presenting cells had contact with GFAP (131). GFAP is mostly expressed intracellularly but can be released into the blood after astrocyte damage where peripheral auto-reactive immune cells might have contact to GFAP and initiate anti-GFAP antibody production (132). On the other hand, peripheral

auto-reactive immune cells could have crossed a pre-damaged blood brain barrier (126, 127).

Additionally, asymptomatic Pug dogs with the high-risk MHC II haplotype show low numbers of pro-inflammatory CD4+ cells in peripheral blood as well as high plasma levels of the anti-inflammatory chemokine IL-10 (128). This might be a compensatory mechanism of the peripheral immune tolerance to keep controlled auto-reactive immune cells. Failing mechanisms may lead to clinically apparent NME.

A genetic predisposition to autoimmune diseases may suggest early onset, although this assertion is only partially accurate. SRMA (a suspected immune-mediated meningitis) typically occurs between 3–18 months of age, while Pugs exhibiting NE typically manifest initial signs of central nervous system dysfunction around 18 months of age on average (53, 97). Other breeds susceptible to NE generally present signs at a slightly older age, ranging from 2 to 4 years (4). Dogs affected by GME tend to be even older, with an age range of 4–8 years, and Australian Shepherds are even diagnosed in their senior years (4, 100).

This can be explained by the theory of multistep pathogenesis of autoimmune disease, which includes an explanation of delayed stochastic penetrance, where physiologic mutations in T-cells might lead to auto-reactive cells (114): physiological and unphysiological activation of lymphocytes by antigens triggers clonal lymphocyte proliferation. In this process, T-cell receptors can change from one T-cell generation to the next. Physiological mechanisms of somatic recombination, gene conversion, and somatic mutation constantly equip the T and B-lymphocytes system with different receptors for detecting antigens (114). Those processes generate random rearrangements of gene segments and result in novel amino acid sequences in the antigen-binding regions of immunoglobulins and T-cell receptors to be equipped for novel antigens (114). This increases the possibility that the immune system can detect a wide variety of external antigens. However, more than half of all antigen receptors generated randomly through somatic recombination also possess the capability to recognize self-antigens (114). Consequently, increased lymphocyte activation increases the risk of auto-reactive memory cells that accumulate with advancing age. This partly amplifies the susceptibility to developing autoimmune diseases with increased age.

Due to the overlap of signalment, clinical signs, and histopathological findings, it seems reasonable to talk of MUO as a disease spectrum rather than an umbrella term summarizing different diseases. In addition, it seems reasonable to include all suspected autoimmune CNS diseases and not only GME and NE as some authors do at the moment (4). Classifying the MUO spectrum should also include breed and age of onset rather than histopathological confirmation alone.

9 Immunological features of MUO

The immunological properties of MUO remain poorly understood. Frequently, studies tend to group various histological subtypes under either MUO or the broader category of inflammatory brain disease (133–135), complicating the assessment of distinctions between MUO subtypes.

When considering lymphocyte population, GME exhibits a mixed pattern involving both B and T-cells, whereas NE is predominantly T-cell-driven (107). CD3+ cells in the CNS of NE-affected dogs produce interferon (IFN)-gamma, contributing to neuronal necrosis in NE (107). Here, a recent study suggested a mild potential superiority of cyclosporine add-on over other add-on therapies when it comes to prognosis in dogs with NE (60). This could be attributed to cyclosporine reducing IFN gamma (136).

In the CNS parenchyma of dogs with GME, a T-helper (Th) 2-dominated immune response is observed (107). Cluster of differentiation (CD)3+ cells in the CNS of GME-affected dogs produce interleukin (IL)-21, IL-17, and IL-4 (107). Monocytes or microglia in GME release IL-17 within the CNS parenchyma (28, 107). IL-21, a type I cytokine produced by T-cells and natural killer T-cells, inhibits the maturation and function of bone marrow-derived dendritic cells (137). However, IL-21 might act as a "double-edged sword" with both stimulatory and suppressive potential, depending on the context (137).

Immunohistochemically activated astrocytes are visible in all subtypes of MUO (138). In close proximity, anti-Glial fibrillary acidic protein (GFAP) antibodies can be found, which are also present in CSF and serum (129, 139, 140). Although anti-GFAP antibodies can also be found in low levels in dogs with brain neoplasia and other encephalopathies, there is a high possibility that they are involved partly in the pathogenesis of MUO (141). This might give a hint to the occurrence of activated auto-reactive B cells and could potentially trigger autoimmune diseases. Notably, anti-GFAP antibodies can also be detected in asymptomatic pugs (129).

Asymptomatic pugs at risk of developing NME due to a specific MHC II haplotype already show variation in their immune system before clinical signs are evident: They exhibit low numbers of CD4+ cells in peripheral blood (128). CD4+ T-lymphocytes play a pivotal role in antigen recognition (142). Furthermore, they communicate with B-lymphocytes, guiding the production of antibodies (143). Moreover, high plasma levels of the anti-inflammatory chemokine IL-10 are present in asymptomatic pugs with the NME risk MHC II haplotype (128).

9.1 Interleukin-31 in MUO

In dogs with MUO, we have identified significantly increased serum levels of IL-31 (Figure 1), a pro-inflammatory cytokine produced by Th2 cells (144). This finding was not observed across all cases: Dogs with elevated IL-31 that had histopathological phenotyping, particularly suffered from GME. Conversely, dogs with infectious meningoencephalitis did not demonstrate elevated IL-31 levels (144).

IL-31 plays a significant role in autoimmune diseases, particularly in human MS, where increased serum levels are prevalent (145). Treated patients with MS displayed a noteworthy reduction in IL-31 serum levels (145). Therefore, IL-31 warrants consideration as a potential prognostic marker for therapy when

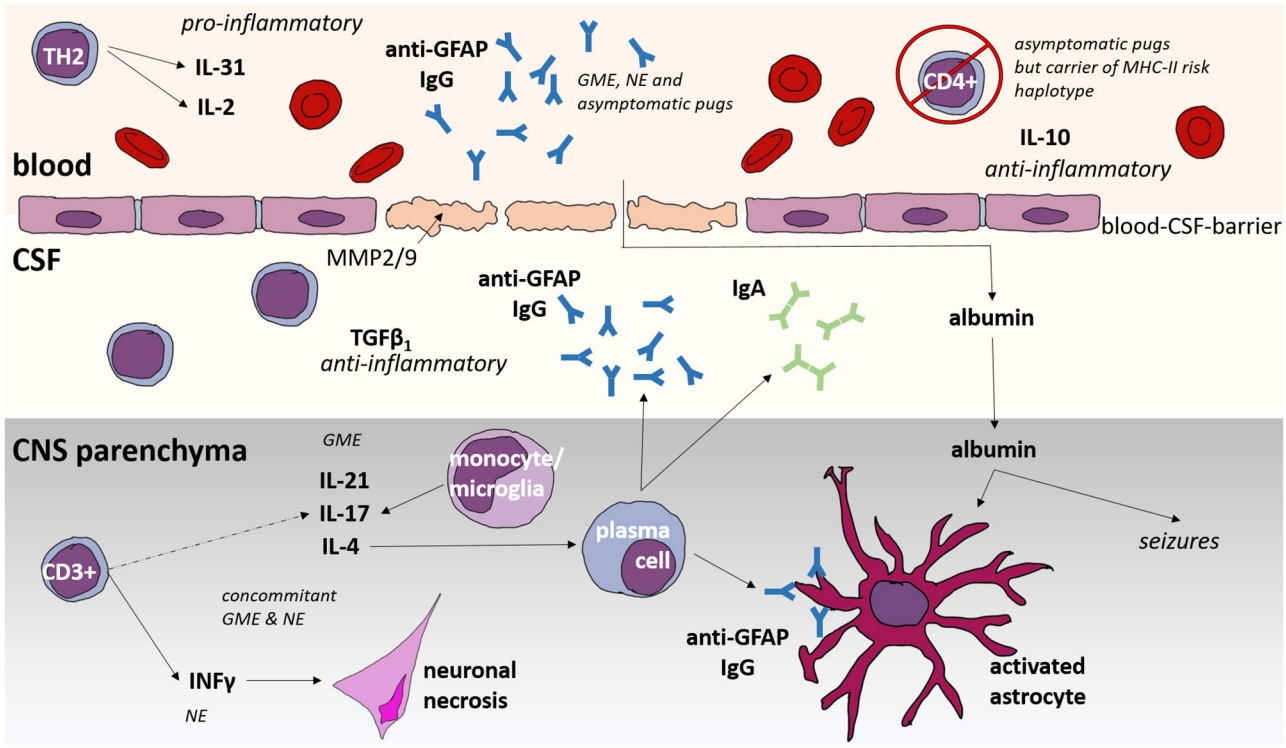

FIGURE 1
Immunological features of Meningoencephalitis of unknown origin (MUO) in dogs. GME, granulomatous meningoencephalomyelitis; NE, necrotizing encephalitis; CNS, central nervous system; CSF, cerebrospinal fluid; IL, interleukin; GFAP, glial fibrillary acid protein; Ig, immunoglobulin; MHC, Major Histocompatibility Complex; CD, cluster of differentiation; TGF, tumor growth factor; INF, interferon.

assessing the progression of MS. Due to the retrospective nature of our IL-31 study and the limited case number, evaluating whether dogs with MUO under treatment exhibit normalized IL-31 levels was not feasible (144).

Exploring the correlation between IL-31 levels and clinical signs, as well as the development of clinical signs depending on IL-31 levels, would be highly informative for future investigations. Additionally, pharmacological blockage of IL-31 might be a new therapeutic strategy to investigate. Lokivetmab is a monoclonal antibody against canine IL-31 and is currently approved for the treatment of itching in atopic dermatitis in dogs (146). It decreases clinical signs of pruritus within 3 h, and the effect of a subcutaneous injection lasts 28–48 days depending on the dosage (146). Long-term studies on healthy dogs showed no side effects beyond those of an unspecific reaction to any subcutaneous injection (147). Therefore, investigation into the clinical effectiveness of Lokivetmab against MUO could be worthwhile.

9.2 Blood-brain-barrier in MUO

The blood- and CSF-brain-barrier (BBB) serves as a highly regulated interface separating the CNS from the peripheral circulation and controlling the exchange of molecules to maintain CNS homeostasis (148). Comprising endothelial cells, pericytes, and astrocytes, the BBB relies on the pivotal role of astrocytes in its formation and maintenance by providing secreted factors that lead to the formation of strong tight junctions (148).

In neurological diseases, the BBB undergoes changes caused by phenotypical alterations in astrocytes amongst others, leading to increased permeability (149–151). This breach allows the extravasation of leukocytes, red blood cells, and plasma proteins into the CNS, as observed in murine experimental autoimmune encephalomyelitis, human MS, and canine MUO (107, 127, 152–155).

In cases of MUO, the BBB is compromised in the majority of dogs (156). Despite damage to astrocytes, the upregulation of MMP-2 contributes to the disruption of the blood-brain barrier (157). Glucocorticosteroid treatment proves effective in restoring the integrity of the blood-brain barrier by inducing the production of MMP inhibitors in SRMA (157) and this might also be the case in MUO.

Dogs with MUO show high levels of albumin in CSF and a high albumin cerebrospinal fluid/serum-quotient (QAlb) (158). As albumin is mostly produced extrathecally by the liver, it may serve as a marker for BBB damage (159).

The Reibergram, utilizing the serum:CSF ratio of albumin and correlating it with the serum:CSF ratio of biomarkers, provides a valuable tool for assessing the integrity of the BBB and determining

whether a molecule was produced intrathecally or extrathecally (160). The hyperbolic curve QLim(IgA) = $0.13\sqrt{((QAlb)^2 + 11.9*10^{-6})} - 1.01*10^{-3}$ describes the upper reference value of the IgA serum:CSF ratio in correlation to the severity of BBB dysfunction (158). An autofill Excel spreadsheet for easy calculation and graphical evaluation of IgA and albumin ratios is available as supplemental data on the paper's journal homepage (158): https://onlinelibrary.wiley.com/action/downloadSupplement?doi=10.1111%2Fjvim.16601&file=jvim16601-sup-0001-Supinfo.zip.

The extravasation of albumin, in particular, can trigger the expression of proinflammatory cytokines, affecting the ability of astrocytes to maintain electrolyte homeostasis (161–164). This scenario may render neurons more susceptible to glutamate excitotoxicity, potentially causing seizures and exacerbate neuroinflammation (161, 165). Furthermore, albumin induces the production of CX3CL1, a chemokine that attracts CD4+ cells (163, 166).

Immunohistochemically activated astrocytes are visible in all subtypes of MUO (107, 167). In serum of Pug dogs with NME, GFAP is detectable in increased amounts (168) and anti-GFAP antibodies can be found in CSF and serum (129, 140, 141). Anti-GFAP antibodies can also be detected in asymptomatic Pug dogs (129), which might imply that the BBB is compromised before clinical signs become evident, allowing contact between self-reactive B cells and the brain, leading to the production of antibodies against astrocyte components. It is conceivable that autoimmunity has already started in these dogs, but anti-inflammatory mechanisms may be preventing the outbreak of NME, thereby maintaining their asymptomatic status.

If activation and destruction of astrocytes and BBB function are the hen or egg in MUO etiopathogenesis remains unclear at the moment. But it is almost evident that the combination of BBB damage, extravasated albumin, and impaired astrocyte function might be a major self-perpetuating vicious circle (Figure 1), which might be one of the major key points to be addressed in the future. Interrupting this circle is also one of the mechanisms of action of prednisolone: Glucocorticosteroid treatment proves effective in restoring the integrity of the blood-brain barrier by inducing the production of MMP inhibitors (157). Additionally, Telmisartan was reported in dogs with idiopathic epilepsy to possibly restore potential BBB damage (169) and could therefore be considered as future treatment option in MUO.

10 Further investigations: requirements and opportunities

Present treatment modalities, heavily reliant on long-term glucocorticosteroid application, may result in iatrogenic hyperadrenocorticism, adversely impacting the QoL of the pets and their owners (13, 47, 48). Attempts to decrease corticosteroid side effects through rapid dose tapering frequently lead to disease recurrence, necessitating additional immunomodulatory drugs (40, 57, 170). However, treatment efficacy remains suboptimal, marked by frequent relapses or insufficient clinical improvement (9).

A deeper understanding of the etiology of MUO is imperative for advancing therapeutic strategies. Critical to this advancement are multicenter studies aimed at unraveling the etiology of inflammatory CNS diseases, coupled with double-blinded multicenter treatment studies. Multicenter studies, by increasing the number of animals involved, allow for more precise examination of individual subgroups and factors such as differences in clinical signs, signalment, and epidemiology can be evaluated. Additionally, large numbers of animals enable the specific examination of homogenous groups with distinct features, such as different breed and age. A consensus on minimal diagnostic criteria is crucial, making multicenter retrospective studies challenging. Clear diagnostic criteria, including breed and age, preferably including biomarkers, are essential for multicentric studies.

11 Suggested polythetic approach for MUO spectrum

For the future, a patient-specific therapy would be desirable. Tailoring treatment to the individual patient's needs could enhance the effectiveness of interventions while minimizing potential side effects. Therefore, it would be beneficial to establish a multifaceted classification system including more than histopathological findings alone. The author suggests conceptualizing the multidimensional spectrum of MUO with a polythetic approach (Figure 2).

"Polythetic" means that members of a group share a subset of characteristics but not necessarily all of them (171, 172). In other words, there is a certain degree of variability in the traits exhibited by the individual within a particular category. This could mean for MUO, that it might be characterized by multiple clinical signs, examination findings, and biomarkers, but not every listed sign or finding is required to diagnose the condition in a particular animal. It might include age of onset of clinical signs, breed, quality and quantity of clinical signs, MRI and CSF findings, severity of BBB damage, serum IL-31 levels and different histopathological features.

For example, different therapeutic options could be tailored to individual patients based on their specific manifestations across various dimensions within the polythetic approach. For example, dogs with a mostly necrotizing variant of MUO might benefit from treatments targeting IFN-γ inhibition, while those with elevated IL-31 levels could respond better to anti-IL-31 antibody therapies. Similarly, dogs with impaired BBB function may benefit from treatments aimed at restoring BBB integrity, which need to be developed in the future.

Furthermore, the polythetic approach may enable us to classify MUO subtypes more precisely than current histopathological methods, helping to develop for example more specific diagnostic biomarkers. This approach could help reveal relationships and patterns that are not apparent with our current understanding, leading to a better understanding of the etiopathogenesis and more targeted and effective treatments.

Using a multidimensional polythetic approach, patient-specific etiopathogenesis enables neurologists to integrate innovative, pathophysiologically based treatments with objective tests—such as stratification biomarkers—to anticipate the potential benefits of

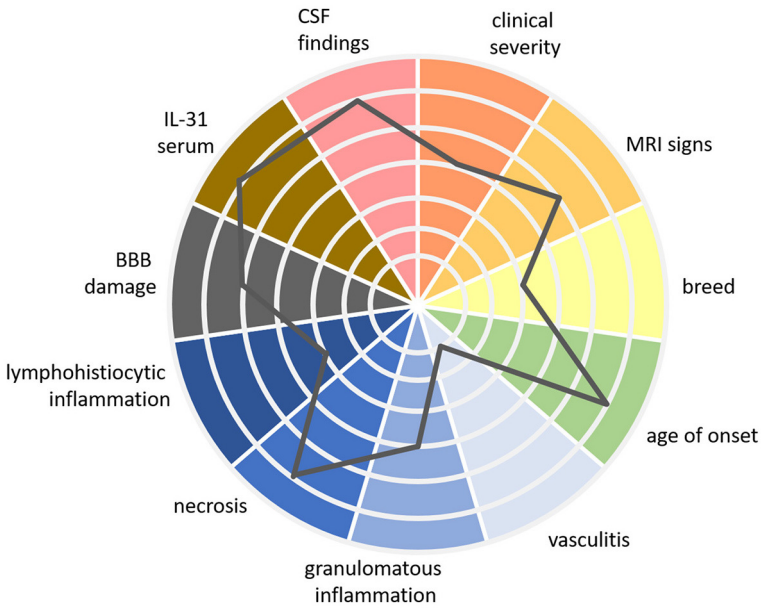

FIGURE 2
Patient specific MUO spectrum using a multidimensional polythetic approach. In this figure, we present a multidimensional, polythetic approach to characterizing patients within the MUO spectrum. Rather than proposing a rigid "classification" in the traditional sense—where patients are placed into well-defined, comparable classes—this approach recognizes the complexity and variability of MUO presentations. It emphasizes personalized assessment by considering multiple clinical and laboratory parameters, with each patient's profile represented as a unique combination of these variables. The gray line in the figure represents the findings for one individual patient, plotted across various axes to show the relative strength of each parameter. This figure is a proposed visual example based on current knowledge, intended to illustrate how different factors can be integrated into a personalized approach. Further research is required to refine the specific values and to better understand the clinical relevance of each marker within this model. While this polythetic approach does not yet define strict classes, it moves toward a framework that could, with additional evidence, lead to a more nuanced understanding of immune-mediated diseases like MUO. MUO, meningoencephalitis of unknown origin; CSF, cerebrospinal fluid; MRI, magnetic resonance imaging; IL, interleukin; BBB, blood-brain-barrier.

distinct treatments for individual cases. This approach allows for tailoring treatment schedules to the patient's specific needs at a given time, reducing the need for long-term prednisolone therapy, minimizing side effects, and improving both treatment efficacy and quality of life.

12 Conclusion

This review lays the basics for forthcoming therapeutic trials, aiming to advance the development of minimally side-effect therapies for patients suffering from MUO.

A polythetic approach that represents the multidimensional spectrum of MUO could adequately address patient-specific needs and potentially decrease adverse effects while improving quality of life. To attain a comprehensive understanding of etiopathogenesis, conducting larger multicenter studies are necessary to recruit an adequate number of patients. For effective multicenter studies, it is crucial to streamline examination procedures across clinics and subsequent analysis.

This review underscores the potential for fostering renewed consensus on diagnostic and classification practices within the diverse spectrum of autoimmune CNS diseases in cats and dogs, serving as a catalyst for prospective treatment studies and encouraging collaborative agreement among researchers and clinicians.

Author contributions

JN: Conceptualization, Project administration, Visualization, Writing – original draft. AT: Conceptualization, Resources, Supervision, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Perspective: Raman spectroscopy for detection and management of diseases affecting the nervous system

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Raman spectroscopy (RS) is used increasingly for disease detection, including diseases of the nervous system (CNS). This Perspective presents RS basics and how it has been applied to disease detection. Research that focused on using a novel Raman-based technology—Rametrix® Molecular Urinalysis (RMU)—for systemic disease detection is presented, demonstrated by an example of how the RS/RMU technology could be used for detection and management of diseases of the CNS in companion animals.

KEYWORDS

Raman, spectroscopy, urinalysis, glioma, brain, tumors

Introduction

The timely and accurate detection of disease affecting the central nervous system (CNS) is key to initiating and providing care for symptomatic patients. Currently, detection of CNS disease relies on data from an adequate medical history, physical and neurologic examinations, imaging and laboratory studies, and response, or lack of response, to treatment. Many laboratory tests rely on the analysis of blood, plasma, serum, and cerebrospinal fluid (CSF) for detection of injury or quantifying organ dysfunction that may indirectly affect CNS activity. Changes in markers of inflammation, such as white blood cell counts, may indicate injury to the structure of the CNS, the presence of infection, or septicemia. These common laboratory procedures are relatively imprecise for localizing disease processes to the CNS or for early detection of disease. While repeated laboratory measurements may change during treatment of disease, they are insensitive in assessing treatment efficacy or rarely used to manage treatment and can be slow to detect outcomes. Urinalysis has not been used commonly in the detection and management of CNS disease, due to the perception that it provides only minimal information reflecting either CNS health or dysfunction.

There is intense interest in human medicine for discovering and using biomarkers of CNS disease. According to the food and drug administration (FDA), a biomarker is "a defined characteristic that is measured as an indicator of normal biological processes, pathogenic processes, or biological responses to an exposure or interventions." This definition was expanded by the National Academy of Science, Engineering, and Mathematics to recognize that biomarkers should demonstrate a response to therapeutic interventions or environmental exposures (1). Much of the research in CNS biomarkers has focused on detection of human neurodegenerative diseases, including forms of dementia, Parkinson's disease, Huntington's

disease, or traumatic brain injury (2–8). Biomarkers of human CNS disease have been used to define the appearance, severity, and progression of disease, and can be a valuable tool in development of drugs for amelioration of disease (1). It is well-recognized that the presentation and progression of disease is patient-specific and that biomarkers may not be useful for the detection and management of disease in all patients. Likewise, lengthy longitudinal studies are frequently needed to demonstrate that specific biomarkers accurately define the trajectory of disease and treatment responses. The cost of developing biomarkers for human CNS disease detection/management is very high—tens of millions of dollars. Recent advances in the development of blood and/or CSF biomarkers suggest they will move from research laboratory studies to clinical use within the next decade, overcoming current limitations of accuracy and cost.

In perspective: research and clinical use of CNS disease biomarkers for animal patient management lags significantly behind research/use for human patient management. Economic and technological considerations will continue to limit development of biomarkers specifically for clinical use in animals, although the use of translational animal models may provide opportunities to apply "human biomarkers" as surrogate "animal biomarkers."

Given the limitations of current standard clinical laboratory testing, and lack of availability of relevant biomarkers for animal CNS disease, there is a need for the development and/or use of non-invasive or minimally invasive, accessible, and affordable technologies for improving patient management and outcomes. In perspective: Raman spectroscopy (RS) of biological samples may meet this need.

Raman spectroscopy basics

RS is a mature, well-studied, and powerful technology that has been applied to analysis of the chemical composition of a wide variety of solids and liquids, including biological specimens (9–18). The analysis can be performed quickly (samples scanned in seconds) on either solids or liquids. Sample volumes required for analysis are small—1 mL of liquids, a few milligrams of most solids—and samples do not need to be chemically-modified for analysis. Raman spectrometers are readily available from commercial sources, are very durable (lifecycle of years is common), have a small footprint (cell-phone size), inexpensive ($5 K–25 K), and operate in ambient conditions typical in medical clinics.

Irradiation of molecular mixtures (e.g., biological fluids like urine or foodstuffs) with wavelength-specific laser energy, produces weak polarization shifts from deformation/relaxation of chemical bonds in hundreds of distinct molecules in the specimens. Bond chemistries, as opposed to individual molecules, are resolved on resulting spectra; however, these are often linked back to biological molecules (e.g., amino acids, carbohydrates, etc.) using spectral libraries (9, 10). To demonstrate the distinguishing capabilities of RS, representative Raman spectra of urine specimens and the urinalysis standard Surine™ from our recent canine study (19) are shown in Figure 1. These were produced from urine samples from healthy dogs and from dogs with brain tumors (including meningioma, glioblastoma, astrocytoma, or oligodendroglioma) or lymphoma. Colored lines are average spectra from all samples of that type collected. Gray shaded regions represent the range observed at each wavenumber. Yellow highlighted regions demonstrate where differences in the spectra were recognizable by the naked eye. It is easy to see that these samples all share a common base level chemical composition (i.e., the unshaded regions), with important spectral differences (i.e., yellow highlighted regions). Thus, a Raman spectrum can be used to not only determine the broader type of fluid (i.e., analytical standard or urine) but more detailed information (i.e., the changing urine metabolome). Without knowing the exact chemicals that give rise to the differentiating peaks in the Raman spectra, we can treat each spectrum as a spectral "fingerprint" of that sample. Analysis of several different types of samples (i.e., urine samples from different patients) can give rise to a large spectral library. This becomes useful when one wishes to know if a disease/metabolic condition is present. Multivariate statistical methods are used to match the Raman spectrum of the new unknown sample to the sample it most closely resembles in the database. We used this strategy in several case studies (19–31).

RS chemometric analysis

The differences between the samples in Figure 1 are clear in some cases (e.g., between 1,200 and 1,425 cm^{-1}), but this is not always the case. For example, the differences between the spectra of brain tumor patients and healthy dogs are difficult to discern around 800 cm^{-1} in Figure 1. Often, computer algorithms are needed to discover key differences in spectral fingerprints in a procedure called "chemometric analysis." Given that the Raman spectra of other complex biological fluids, such as plasma, serum, whole blood, and cerebrospinal fluid (10, 11, 18, 31–35) are composed of literally thousands of peaks and valleys (at points designated as wavenumbers), the chemometric fingerprinting approach is likely to be effective with these.

Chemometric comparisons of spectral fingerprints can be done by a wide variety of statistical, machine learning (ML), and artificial intelligence (AI) models (36–40). Multivariate analysis of variance (MANOVA) modeling of urine fingerprints in Figure 1 is shown in Figure 2A. MANOVA, a supervised modeling technique, seeks differences between groups of spectra and exploits those to create clusters. The process of MANOVA model-building is referred to as "training," and these results are shown in Figure 2. Supervised models require "testing," where spectra unknown to the model are processed and model predictions are compared with known results. For urine screening, the accuracy, sensitivity, specificity, positive-predictive value (PPV), and negative-predictive value (NPV) are often used to describe model performance (41). Much can be learned from MANOVA model training. In Figure 2A, Raman spectra of Surine™ were separated along Canonical 1, the primary MANOVA axis. This was the "easiest" separation, consistent when comparing Surine™ urine control spectra to the other urine spectra in Figure 1. Lymphoma urine spectra were separated along with second MANOVA axis (Canonical 2), leaving spectra from healthy dogs and dogs with brain tumors clustered together. Thus, urine of lymphoma patients is relatively easy to identify by the model. This left urine from healthy dogs and those with brain tumors clustered together. Separating these required a new model, where only these two groups were included. The MANOVA training model is shown in Figure 2B. However, when tested and validated with leave-one-out cross validation, accuracy >90% was obtained, with 100% sensitivity and >83% specificity for identifying a urine sample from a dog with brain tumor. We used a small number of samples in each group for this analysis, as a preliminary proof-of-concept - that needs more validation with larger datasets. However, we show how

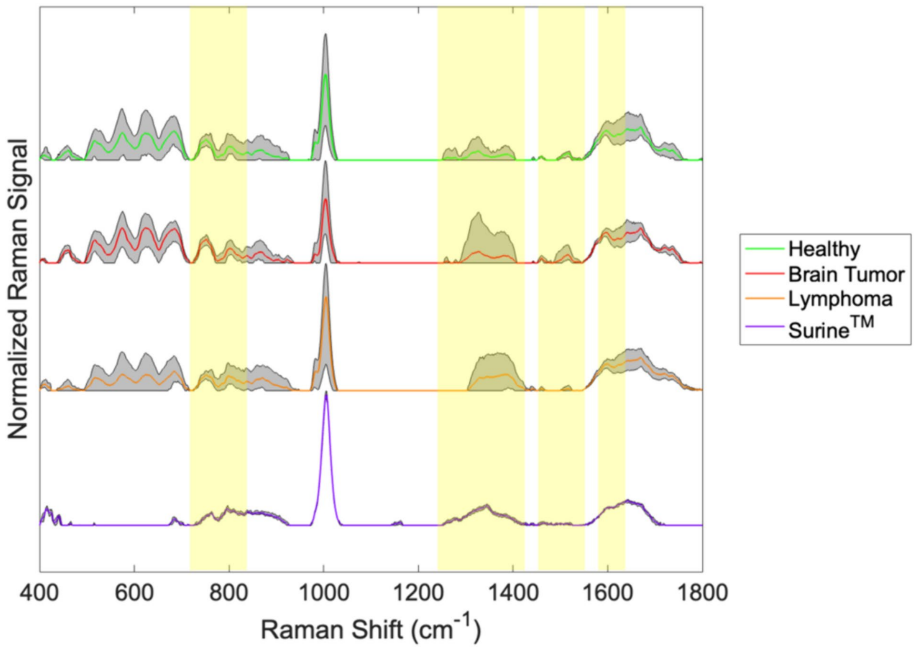

FIGURE 1
Spectral fingerprints for canine urine produced from ISREA baselining and processing raw Raman spectral data.

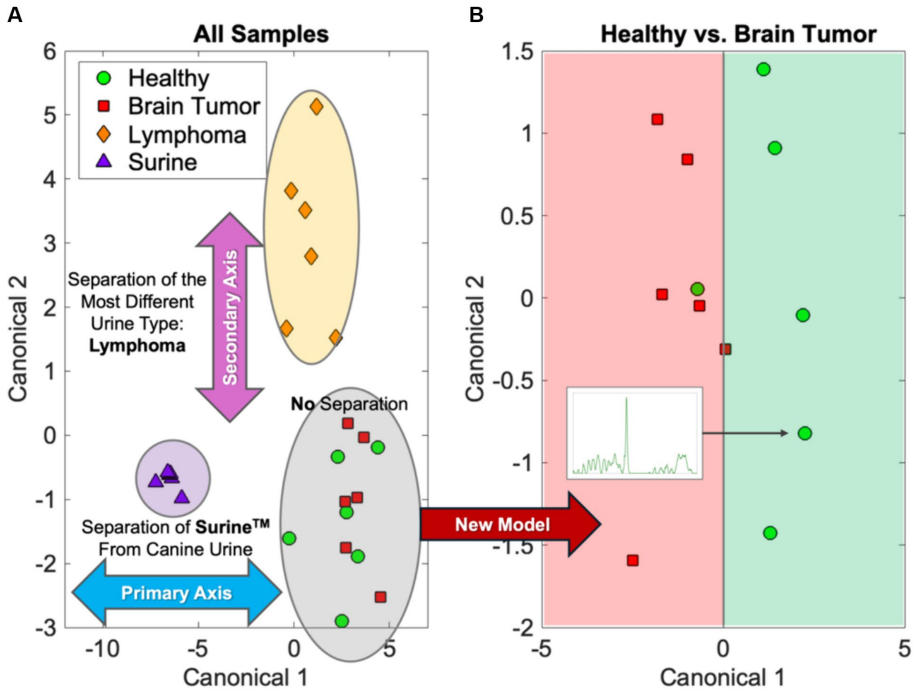

FIGURE 2
MANOVA modeling of the urine fingerprints in Figure 1. **(A)** The initial separations of lymphoma and Surine samples and **(B)** separation of the more similar healthy and brain tumor samples.

multiple MANOVA models can be used to separate out and classify very different samples first and then dedicate models to focus on samples that have few differences from one another.

In perspective: As databases of Raman spectra of biological molecules improve in breadth and availability, our ability to cross-reference more regions of the spectral fingerprint and confirm analytical results with mined literature data will improve. The procedure of spectral fingerprint reduction with computational and statistical techniques also offers the opportunity to identify new molecular contributors and specimen metabolomic differences due

to the presence of a disease. These new metabolomic markers can be verified through other analytical means to further validate the fingerprint.

Medical applications of RS, with special reference to CNS disease

The value of RS in detection and management of human diseases has become well-recognized in the past decade, in studies ranging from cancer detection (18, 42–48), to cardiovascular dysfunction (49), and as an aid to theranostics (50). The interested reader is encouraged to delve into the substantial medical literature on uses of RS for disease detection and management.

There has also been substantial interest in using RS for detection of CNS disease. Ranasinghe et al. (3, 4) published a comprehensive review of the uses of RS and "liquid biopsy" techniques for studying human brain disorders, specifically neurodegenerative diseases such as Alzheimer's, Parkinson's, and Huntington's disease(s), amyotrophic lateral sclerosis, and traumatic brain injury (TBI), using blood, plasma, CSF, tears, and saliva samples. This excellent review also discussed chemical- and nanoparticle-RS signal enhancement techniques for improving detection of small molecules in biological fluids. Limitations in RS analysis/technology, including problems associated with reproducibility, sample composition (mixing) variation, quantification of molecular species in complex biological fluids like blood and CSF, interfering chemical and physical phenomena, and data analysis are reviewed in their work. Chen et al. (6) similarly reviewed the uses of RS for detection of neurodegenerative diseases, while also discussing the societal burden posed by these diseases in aging human populations. Terrones et al. (5) discussed the use of RS and tagged tracer molecules for studying the pathogenesis of brain disorders (neurodegenerative disease, TBI/ischemia, and CNS neoplasia), as well as for assessing the localization and kinetics of facilitated drug delivery. In perspective: while this work in human patients demonstrates that the applicability of RS to the study of brain disorders, it is limited to clinical research applications that are not directly applicable to veterinary neurology, given profound differences in the spectrum of spontaneous disease between human and companion animals.

Recently, RS has been used to detect and classify brain neoplasms (42, 51). Novel sensors, imaging modalities, and surface enhanced RS (SERS) for the detection and management of gliomas were reviewed by Thenuwara et al. (52). Several studies reported intriguing results of scanning of brain neoplasm biopsies or serum from patients with tumors. Riva et al. (53) scanned fresh (not fixed or frozen) biopsies from 63 patients undergoing surgical resection of gliomas. Using RS and ML, they were able to discriminate between healthy, non-neoplastic tissue and tumor tissue with an accuracy of 82% and noted that 19 unique Raman "bands" were the basis for discrimination. They, and others (51, 54–56), suggested that RS probes could be used intra-operatively to define tumor margins during resection—always a significant challenge with gliomas. Zhang et al. (57), using RS, compared serum sample from healthy individuals ($n = 86$) and patients with glioma [high-grade glioma (HGG) $n = 75$, low-grade glioma (LGG) $n = 60$]. They were able to classify, based on differences in serum Raman spectra, differences between healthy, HGG, and LGG groups with an accuracy of 94.12%. In perspective: these studies clearly have application to the practice of veterinary neurology and neurosurgery.

Molecular urinalysis

Urine is a readily available complex fluid that can easily be obtained non-invasively or with minimal invasion (cystocentesis, catheterization) in companion animals. It is the product of systemic physiologic and pathologic processes, metabolism, and renal function—in essence, a dynamic "liquid biopsy" of the body. Studies have shown that normal human urine contains more than 2,500 separate chemical entities, many of them present in micromolar concentrations (11).

The composition and physical properties of urine in healthy people and animals varies widely each day. Changes in urine volume and urea content, for example, are predictably related to many physiologic/metabolic factors including the state of hydration, water intake, physical activity, and diet. The presence of miniscule amounts of hemoglobin/erythrocytes, sloughed genitourinary tract cells, and flora/fauna from the lower urinary tract is recognized and accepted as part of normal urine composition in free-catch urine specimens.

Systemic and genitourinary tract diseases change the volume, physical properties (e.g., pH, specific gravity, conductivity, color, turbidity, viscosity), suspended sediments (e.g., cells, minerals, casts), and other components of urine. The presence of excessive amounts of glucose and protein in urine, for example, invariably raises concerns about the presence of diabetes mellitus and/or renal pathology. These changes can be readily detected by routine urinalysis which includes assessment of the physical properties, sediments, cytology, and chemical composition (usually with a point-of-care dipstick "dry chemistry" analysis) (58, 59).

Over the past decade, "molecular urinalysis" involving urine metabolomics and urine biomarkers have been increasingly used in clinical research settings for disease detection. Mass spectrometry (MS), liquid/gas chromatography (LC/GC), nuclear magnetic resonance (NMR), capillary electrophoresis (CE), and kinetic nephelometry methods, have been used for detection of changes in urine analytes associated both with both normal physiology/metabolism and with disease states. These high technology approaches have had limited clinical translation. Magalhaes et al. (60) used CE/MS to identify polypeptide patterns in urine of human patients with kidney disease and diabetes. Patterns identified could not, however, be correlated with specific molecules (biomarkers) of physiological and pathophysiological significance. Darshi et al. (61) used MS to study diabetic kidney disease (DKD)-related metabolomic alterations. They noted their observations could not be readily applied to human patient management given the diverse spectrum of diabetes mellitus, chronic kidney disease (CKD), associated co-morbidities, and lack of correlation to standard metrics to ongoing, progressive disease trends, and patient demographics. In perspective: MS-based metabolomics are not used for routine patient care. The complexities of many systemic diseases, and the need for collection of large datasets to validate such technology-intensive methods, makes their use unlikely and cost-prohibitive—certainly out of the reach for most animal owners and their healthcare providers. The expense of purchasing and maintaining laboratory MS-based technologies, expertise required for operation and interpretation of results, lack of assay validation with large datasets of normal and abnormal specimens, will continue to limit clinical use.

Rametrix® molecular urinalysis

We recognized the limitations and challenges associated with MS-based metabolomics and biomarker assays. To address these, we invented and extensively validated a RS technology—Rametrix® Molecular Urinalysis (RMU) (19, 20, 22, 24, 28, 30). The basic ideas behind RMU are demonstrated in Figures 1, 2 and involve additional ML/AI models to discover disease-specific spectral fingerprints in Raman spectra of urine. As previously stated, RMU technology, capitalizing on the many RS features previously described has these advantages over other molecular urinalysis technologies is rapid (<15 s per sample), inexpensive, requires small sample volumes (*ca.* 1.5 mL), is non-destructive of samples, requires no sample processing, can be done on thawed, frozen samples (which are stable for months), can be adapted to point-of-care testing and automation, and draws on data from our extensive RS databases.

We have applied RMU to study urine molecular composition of healthy human volunteers (27), human patients with CKD 4–5 (30), DKD (23), chronic Lyme disease (29), COVID19 (24, 26), and dogs with cancer (19), among other health and disease states. As one component of our recent study of dogs with cancer (unpublished data), we analyzed the urine of eleven (11) dogs with a variety of CNS neoplasms (above), with RMU. Although the number of patients with each tumor histotype was small, the spectral fingerprints of all tumors, considered as representative of brain tumors, was significantly different from healthy dogs ($n = 89$), and dogs with other common tumors, including lymphoma ($n = 53$), bladder cancer ($n = 18$), and mast cell tumors ($n = 17$). In perspective: it appears RMU can detect and differentiate canine tumors localized in the CNS.

Discussion

In this Perspective, we have discussed the basics of RS and the application of this powerful technology to disease detection and management, including diseases of the CNS. There has been significant interest and research in applying RS to human neurodegenerative diseases and to detection and management of gliomas. We developed RMU as an RS-based platform and have used it to study both human and animal (canine) disease. We have validated the technology with studies in >4,000 human, >200 canine, and > 250 equine urine samples, collected for clinically-healthy individuals and from those with a variety of diseases. As a next phase of our work, we intend to focus on expanding our studies using RMU for detecting and as an adjunct to managing canine cancer. Specifically, we will determine how useful RMU is for pre-operative detection and post-operative surveillance of CNS malignancies and use it to discover spectral fingerprints other infectious, inflammatory, traumatic, and degenerative disease in companion animals.

Data availability statement

The data presented in the study are deposited in https://github.com/SengerLab/Raman-Scans/tree/Canine.

Ethics statement

The studies involving humans were approved by the Virginia Tech Institutional Review Board. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

JR: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. AS: Conceptualization, Data curation, Formal analysis, Software, Writing – review & editing. RS: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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Conflict of interest

JR, AS, and RS were employed by Rametrix Technologies, Inc. The authors of this Perspective are inventors and developers of the Raman Molecular Urinalysis (RMU) technology described and used in this paper. They have founded a corporation, Rametrix Technologies, Inc. (www.rametrixtech.com) and intend to commercialize the technology for disease detection and management.

Publisher's note

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Hemorrhagic encephalopathies and myelopathies in dogs and cats: a focus on classification

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The prevalence of hemorrhagic diseases of the central nervous system of dogs and cats is low compared to other diseases such as neoplasia and inflammation. However, the clinical consequences can be devastating. Several etiological and localization-based classification systems have been reported for intracerebral and spinal cord hemorrhage or hematomyelia in humans but similar systems do not exist in veterinary medicine. The authors propose an etiologic classification system for both intraparenchymal hemorrhagic encephalopathy and myelopathy following a review of the literature detailing the presentation, diagnosis, therapy, and prognosis of these diseases. A summary of the investigative and therapeutic approach to these cases is also provided.

KEYWORDS

hemorrhagic infarct, hematomyelia, vascular malformation, T2* gradient echo, susceptibility-weighted imaging

Introduction

Hemorrhagic encephalopathies and myelopathies in dogs and cats can have various etiologies and localizations. In human medicine, classifications based on etiology are commonly used and have been subject to change over time. In veterinary neurology, etiological classifications are not specifically agreed upon.

Classification can be important because it may contribute to a better understanding of underlying pathology, facilitate comparability between patient populations at different care facilities (e.g., for research purposes), and improve patient care by guiding diagnostic, therapeutic, and preventative measures.

Classification systems in human medical literature have been developed and employed, providing the opportunity to study both hemorrhagic encephalopathies and myelopathies retrospectively and prospectively with regard to associations with outcomes or effects of therapy.

While many pathological processes may involve some degree of hemorrhage, not all those encephalopathies or myelopathies with macro-or microscopic hemorrhage should be termed 'hemorrhagic encephalopathies/myelopathies' *per se*. For the purpose of this review, we define hemorrhagic encephalopathies and myelopathies as those encephalopathies and myelopathies where hemorrhage is an inherent part of the primary pathology, (suspected) main cause of the clinical signs, and/or dominant feature on diagnostic imaging or post-mortem examinations.

This review aims to 1/discuss classifications of hemorrhagic encephalopathies and myelopathies in humans, 2/summarize reported etiologies of canine and feline intraparenchymal hemorrhagic encephalopathies and myelopathies, 3/propose simple, easily

applied classification systems based on etiology and localization, and 4/briefly review diagnostic, therapeutic, and prognostic considerations, based on veterinary and human literature.

Hemorrhagic encephalopathies

Etiological classification

Human

In human medical literature, several etiological classification systems have been reported for intracerebral hemorrhage specifically. The three most recently reported of these are chronologically included and discussed below.

- SMASH-U (1)

This classification system was devised to be simple and clinically practical. The six categories of disease are structural vascular lesions (S), medication-associated (M), amyloid angiopathy (A), systemic disease (S), hypertension (H), and undetermined (U). Traumatic and tumor-associated etiologies were excluded. The undetermined (U) category was not specified further. The authors proposing this system evaluated the reproducibility (interobserver agreement, kappa of 0.89) and prognostic value in a retrospective study on 1,013 patients with signs or symptoms of >24 h duration. Several categories had odds ratios significantly different from 1 (either <1 or > 1) for mortality at 3 months post-diagnostic procedures. Based on this, the authors concluded that the classification system as a whole has prognostic value. In the evaluation of this system, the authors did not account for the possibility of more than 1 category and strict definitions were mostly lacking.

- H-ATOMIC classification (2)

This classification system was formed based on the frequency of diagnoses in human medicine and includes 7 categories of disease which are hypertension (H), cerebral amyloid angiopathy (A), tumor (T), oral anticoagulants (O), vascular malformation (M), infrequent causes (I), and cryptogenic (C). This system also does not account for traumatic hemorrhagic encephalopathies, as this etiology was specifically excluded. The authors acknowledged that more than 1 category may apply to a single patient. The authors included three levels of certainty for disease categorization: definite (1), probable (2), and possible (3). For instance, this leads to a classification of T1 for confirmed tumor-associated hemorrhagic encephalopathy. The cryptogenic category was concluded based on the exclusion of the other categories. The 'infrequent causes' category was broad and based on the relatively low prevalence of these causes in humans with hemorrhagic encephalopathies. This category included but was not limited to disorders such as intracranial aneurysms (congenital, mycotic, other causes), venous thrombosis, illegal drug-or alcohol-associated, hypertensive crisis, and pituitary apoplexy. Along with this classification system, guidelines for diagnostic protocols and minimal work-up were published, including definitions of the specific requirements to be assigned to a category of this classification. Notably, the etiologic category of 'oral anticoagulants (O)' is not considered a real cause of hemorrhage in the brain, but rather a risk factor by other authors (3).

- CLAS-ICH (3)

CLAS-ICH stands for 'classification system for intracerebral hemorrhage' and is an imaging-based system. The system was devised by the authors based on recent neuroimaging advancements, recent data regarding the epidemiology (prevalence) of intracerebral hemorrhage, and advancements in cerebral small vessel disease (SVD) phenotyping. The categories were also defined keeping in mind potential clinical relevance, i.e., the system had to be simple and yield outcomes of possible relevance for acute patient management. Finally, categories were defined accounting for overlapping pathogenesis and prognosis that may affect treatment strategies.

Five categories are included: arteriolosclerosis, cerebral amyloid angiopathy, mixed small vessel disease (SVD), other rare forms of SVD (genetic SVD and others), and secondary causes (macrovascular causes, tumor, and other rare causes). Traumatic causes are not accounted for and were not included in the study for evaluation of this system. Every patient is scored in each category according to the level of diagnostic evidence: (1) well-defined ICH subtype; (2) possible underlying disease; and (0) no evidence of the disease Unlike the previous two systems, patients are classified for each of the categories. This is based on the level of certainty or 'diagnostic evidence' and graded as well-defined; (1) possible underlying disease; (2) and no evidence of disease (0). This system therefore acknowledges that multiple etiologies may be relevant in a single patient. This system was studied in two cohorts of 113 and 203 patients, respectively. Interobserver agreement was very good to perfect (kappa of 0.86–1.00).

Veterinary literature and classification proposal

According to the veterinary neurology acronym of VITAMIN D, hemorrhagic encephalopathy has been reported for each of the abbreviated categories (Table 1). In the literature documenting these disorders, hemorrhage is often mentioned as an aside. That is, hemorrhage was deemed to be a secondary feature of the primary disorder. The hemorrhage itself may not be responsible for clinical signs or a specific target of treatment. On the other hand, hemorrhage as a secondary feature of another primary disorder may be a pivotal complication with possibly fatal consequences.

As gleaned from the human etiological classification system, traumatic causes are often excluded or seen as an entirely separate and clearly identifiable (from the history or presentation) cause. However, in veterinary medicine, unobserved trauma and a lack of trauma in the history due to a lack of the ability to self-report is much more common. We therefore include trauma as an etiological category in our classification proposal.

For the proposed veterinary classification system, we elected to include the considerations above. As the aim was for the classification system to be simple and easily applied, three broad categories were defined, with sub-categories listed according to the well-known VITAMIN D scheme that veterinary neurologists, neuroradiologists, and neurosurgeons will be aware of (Table 1).

The classification system (Table 2) includes the following categories:

- Primary hemorrhagic encephalopathy

This category includes vascular disorders (e.g., cerebral amyloid angiopathy/beta-amyloid angiopathy) (4–6) (Figure 1), excluding

TABLE 1 Hemorrhagic encephalopathies classified into VITAMIN D categories, including example references.

Vitamin D category	Examples	References
Vascular	Hemorrhagic stroke Hemorrhagic transformation of ischemic stroke Cerebral amyloid angiopathy / beta-amyloid angiopathy	(6, 8, 13, 122–125)
Idiopathic		(7, 12, 33)
Inflammatory-infectious	Viral infections (e.g., canine adenovirus) Feline infectious peritonitis Protozoal encephalitis Bacterial encephalitis	(126)
Inflammatory-immunemediated	Meningoencephalitis of unknown origin	(127–129)
Traumatic	Type of trauma (e.g., traffic accident, bite trauma) With/without cranial vault fractures With/without pre-existing brain disorder	(24, 27)
Toxic/Drug-associated	Lead intoxication Rodenticide poisoning	(130–134)
Anomalous	Vascular malformations	(13)
Metabolic	Various endocrinological disorders (associated with hemorrhagic diathesis or hypertension) Electrolyte imbalance (e.g., hypernatremia)	(32, 135)
Neoplastic	Lymphoma Hemangiosarcoma Glioma	(100, 136–141)
Nutritional	Thiamine deficiency	(142)
Degenerative	Fibrinoid leukodystrophy	(143, 144)
Other	Hemorrhagic diathesis Heat stroke Hypertension-associated	(32, 33, 70, 145–150)

TABLE 2 Veterinary classification scheme of hemorrhagic encephalopathies as suggested by the authors.

Category	Examples
Primary	Idiopathic hemorrhagic stroke Idiopathic hemorrhagic encephalopathy Cerebral amyloid angiopathy/beta-amyloid angiopathy Hypertension associated hemorrhagic encephalopathy
Secondary	Vascular malformations Neoplasia (primary brain tumors or metastatic disease) Vasculitis associated or not with meningoencephalitis (infectious or immune-mediated) Coagulopathies (hemorrhagic diathesis of any cause) Infarcts with hemorrhagic transformation Metabolic/nutritional/degenerative/toxic disorders Heat stroke
Traumatic	Specify type of trauma (e.g. traffic accident, bite trauma) Subcategories: With/without cranial vault fractures With/without pre-existing brain disorder (e.g., hydrocephalus)

vasculitis and malformations (included in the secondary category), hypertension-associated hemorrhage (Figure 2), idiopathic hemorrhagic stroke (i.e., no underlying causes identified) (Figure 3), and idiopathic hemorrhagic encephalopathy. Cerebral microbleeds are included in this category and may be associated with hypertension or angiopathy (microvascular disease). In dogs, they has been associated with proteinuria (7–11). Hemorrhagic stroke would be characterized by acute to peracute onset of clinical signs with localization to any part of the brain and diagnostic or histopathological findings of hemorrhage (12). Idiopathic hemorrhage would include those cases in which hemorrhage is found, but the clinical signs are not reflective of a stroke (i.e., there is no acute to peracute history) and further tests including diagnostic imaging and histopathology did not reveal any specific causes.

Note: 'cerebrovascular accident (CVA)' is a term that is used in literature interchangeably with 'stroke' and may refer to ischemic stroke or hemorrhagic stroke.

- Secondary hemorrhagic encephalopathy

This category includes vascular malformations (13) (Figure 4), neoplasia (primary brain tumors or metastatic disease), vasculitis, meningoencephalitis (infectious or immune-mediated) (Figure 5), coagulopathies (hemorrhagic diathesis of any cause), and infarcts with hemorrhagic transformation (i.e., ischemic stroke with hemorrhagic transformation). Metabolic, nutritional (e.g., thiamin deficiency), degenerative, or toxic (e.g., lead intoxication) disorders and heat stroke may also feature hemorrhagic lesions in the brain.

- Traumatic hemorrhagic encephalopathy

This is separately classified, as mechanical injury to brain tissue and blood vessels results in hemorrhage as a complicating factor. The type of trauma should be specified if possible (e.g., traffic accident, bite trauma). Subcategories include those with or without cranial vault fractures and those with or without pre-existing brain disorders (e.g., hydrocephalus (Figure 6), or brain neoplasia).

Selected examples of some of these categories are included in Figures 2–6.

Localization

Intracranial hemorrhage may be classified based on localization. For completeness, we include all possible intracranial localizations of hemorrhage below.

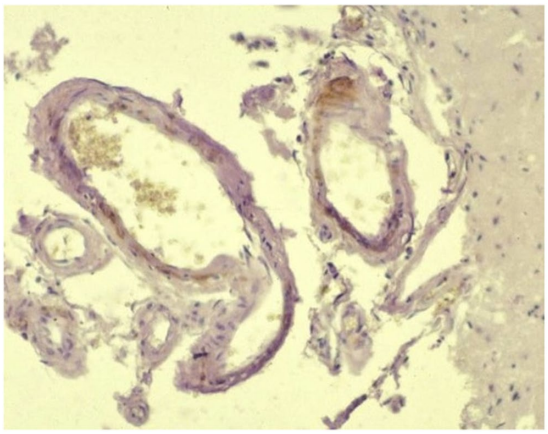

FIGURE 1
Beta-amyloid angiopathy: amyloid deposition in the wall of a leptomeningeal vessel (Avidin–biotin peroxidase complex (ABC) method for beta-amyloid protein, Mayer's hematoxylin counterstain) – courtesy of M. Pumarola, Unit of Compared and Murine Pathology, Department of Animal Medicine and Surgery, Faculty of Veterinary Medicine, Universitat Autònoma de Barcelona, Barcelona, Spain.

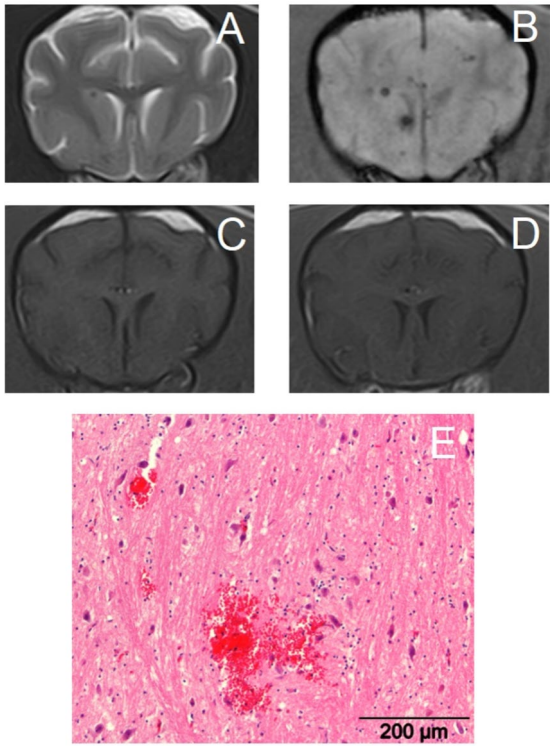

FIGURE 2
Transverse magnetic resonance images of the brain of a 14-year-old crossbreed dog with a primary hemorrhagic encephalopathy – hypertension-associated cerebral microbleeds. All images are at the level of the head of the caudate nuclei. Left is on the right of the images. (A): T2-weighted, (B): Susceptibility-weighted, (C): T1-weighted, (D): T1-weighted post-contrast, (E): photograph of hematoxylin and eosin stained brain tissue with an acute cerebral microbleed – courtesy of W. Bergman, Veterinary Pathology Diagnostic Center, Department of Biomedical Health Sciences, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands.

However, this review focuses on intraparenchymal hemorrhage, the most common localization in small animal clinical neurology. This 'classification system' is not species-specific (i.e., applicable to humans, dogs, cats, *et cetera*).

Intracranial hemorrhage may be located in any of the following locations (12, 14–21):

- Extraparenchymal hemorrhage

  - o Epidural hemorrhage: outside of the dura mater
  - o Subdural hemorrhage: under the dura mater, outside of the arachnoid membrane
  - o Subarachnoid hemorrhage: within the subarachnoid space
  - o Subpial hemorrhage: under the pia mater
  - o Subependymal hemorrhage: under the ependymal lining of the ventricles
  - o Intraventricular hemorrhage: within the ventricular system

- Intraparenchymal hemorrhage: within the brain parenchyma

One, two, or multiple of these localization may be involved at once. This list of localizations can be elaborated even more extensively and details can be added to the localizations mentioned. For instance, for intraparenchymal hemorrhage, one can localize it to specific structures or regions and/or gray versus white matter; and for intraventricular hemorrhage, the specific parts of the ventricular system that are involved should be mentioned.

The value of a localization-based classification lies mainly in the option of surgically addressing the hemorrhagic component itself. For instance, an extraparenchymal hemorrhage or epidural hemorrhage may be cleared via craniotomy, flushing, and suction.

Some of the localizations have not been specifically covered in previous reviews on hemorrhagic brain disorders in small animals but can be found in human literature, particularly in neonates [e.g., subependymal hemorrhage (17)]. Whether or not some of the above localizations have or have not been specifically reported is not particularly relevant as long as readers are aware of the theoretical localizations of the hemorrhagic components. Intraparenchymal hemorrhages are most commonly reported in dogs and cats. Extra-parenchymal hemorrhage in small animals is most commonly the result of trauma (20–22).

Clinical signs

Clinical signs related to hemorrhagic encephalopathies are primarily dependent on the location of the hemorrhage within the brain tissue, the extent of hemorrhage, associated pathology, secondary effects (e.g., mass effect), and time frame (e.g., acute versus chronic phase). Therefore, signs vary greatly and may include abnormal mentation, abnormal behavior, epileptic seizures, cranial nerve deficits, abnormal postures, paresis, proprioceptive deficits, ataxia, hyperesthesia, and abnormal sensation.

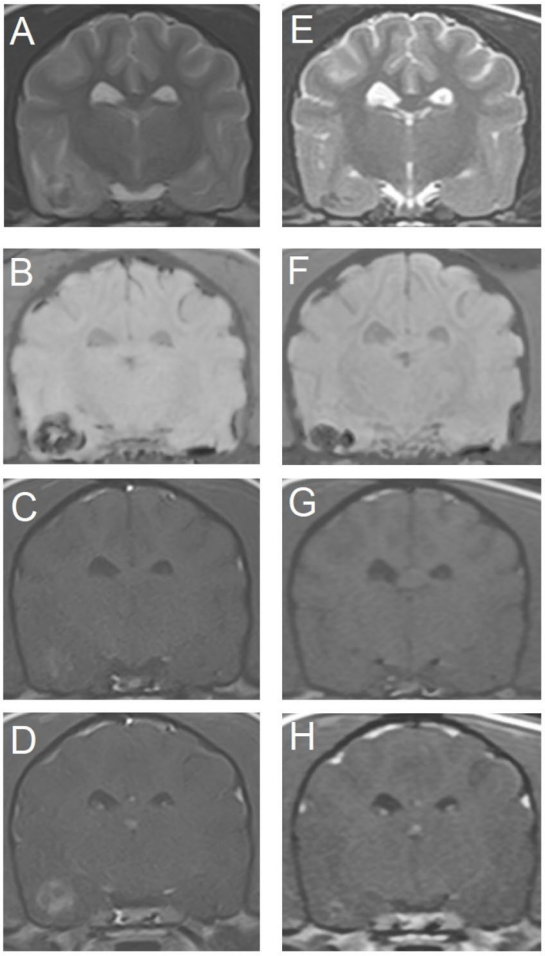

FIGURE 3
Transverse magnetic resonance images of the brain of an 11-year-old miniature Dachshund with a primary hemorrhagic encephalopathy – idiopathic hemorrhagic stroke. All images are at the level of the temporal lobes and caudal aspect of the interthalamic adhesion. Left is on the right of the images. A–D are images from a study 3 days after peracute onset of clinical signs. E–H are images from a follow-up study performed 4 weeks later. (A,E) T2-weighted. (B,F) Susceptibility-weighted. (C,G) T1-weighted. (D,H) T1-weighted post-contrast.

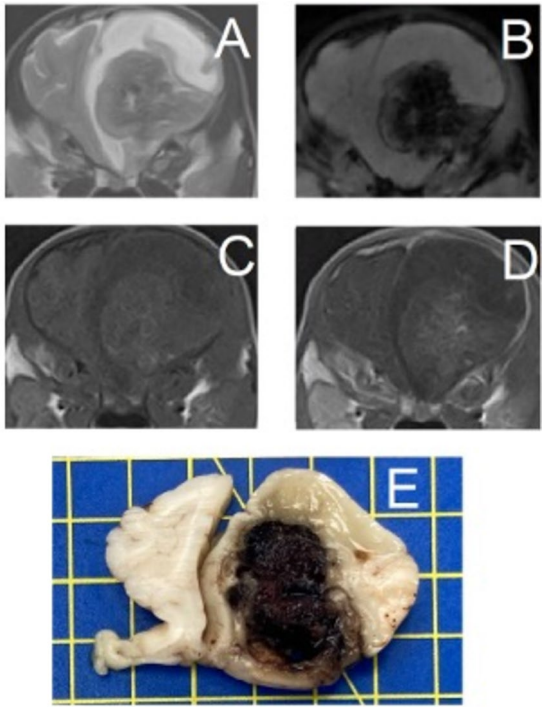

FIGURE 4
Transverse magnetic resonance images and formalin-fixed transverse slab of the brain of a 2-month-old Spanish Waterdog with a secondary hemorrhagic encephalopathy – secondary to a ruptured cavernous hemangioma. All images are at the level of the frontal lobes just rostral to the septum telencephali. Left is on the right of the images. (A) T2-weighted, (B) Susceptibility-weighted, (C) T1-weighted, (D) T1-weighted post-contrast, (E) photograph of formalin-fixed slab of brain tissue (yellow squares are 1×1 cm) – courtesy of N. Ankringa, Veterinary Pathology Diagnostic Center, Department of Biomedical Health Sciences, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands.

Diagnostic considerations

In general, the history will be instrumental in excluding the traumatic category of hemorrhagic encephalopathy. If there is no owner or no history of trauma, trauma cannot be excluded immediately. Therefore if in doubt, this possibility should be taken into consideration for both diagnostic decision-making and treatment.

For most cases of hemorrhagic encephalopathies, diagnostic imaging will be necessary for *in vivo* diagnosis. Magnetic resonance imaging (MRI) would be the modality of choice in the majority of cases, excluding acute traumatic cases, in which computed tomography (CT) offers numerous advantages, including shorter scan time, no need for anesthesia (i.e., sedation or even non-sedated studies can be performed if patients are immobile), and better sensitivity for cranial vault fractures (23). Nevertheless, MRI has been shown to have prognostic value after traumatic brain injury in dogs (24).

In the human medical literature, the authors of the H-ATOMIC classification specifically detail diagnostic criteria and recommendations (2). These can also be considered for veterinary patients, to accurately classify hemorrhagic encephalopathies as well as to determine the best course of action for that patient. These broadly include (2, 25, 26):

- Neuroimaging (see above, CT versus MRI; consider angiography, especially for vascular malformations; consider follow-up imaging studies). For MRI studies, include T2*-weighted or susceptibility-weighted-imaging sequences (Figures 2–6). Follow-up imaging studies can be crucial to exclude or confirm suspicions of underlying disorders, such as neoplasia (Figure 3). The reader is referred to Arnold et al. for specific considerations for MRI studies (11).
- Blood tests (e.g., complete blood counts, differentiation, biochemistry (electrolytes, glucose, urea, creatinine), coagulation profiles (thromboelastography and D-dimers), endocrine tests)
- Cardiac ultrasound and electrocardiogram
- Thoracic and abdominal imaging studies (radiographs, ultrasound, CT – e.g. staging or looking for primary neoplastic disorders)
- Cerebrospinal fluid analysis (when no contraindications are present, preferably after diagnostic imaging and when hemostatic disorders are excluded)

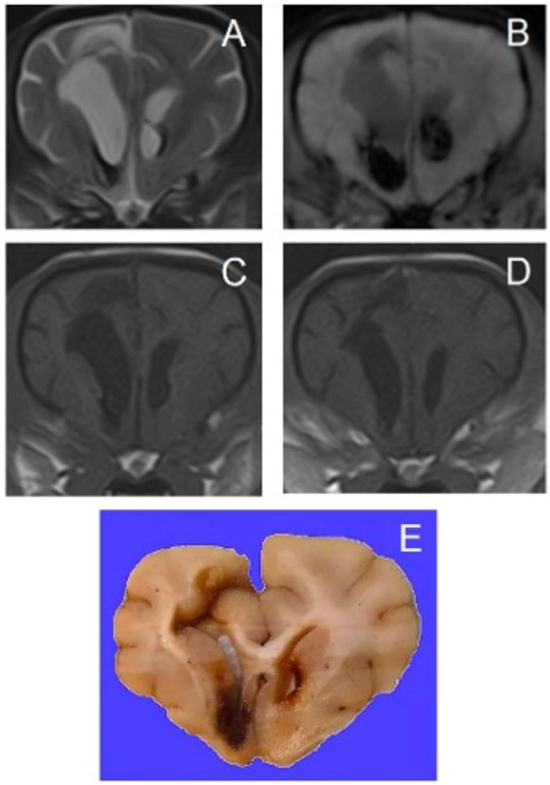

FIGURE 5
Transverse magnetic resonance images and formalin-fixed transverse slab of the brain of a 7-year-old Pomeranian with a secondary hemorrhagic encephalopathy – secondary to multifocal, non-symmetrical, lympho-plasma-histiocytic, necrotizing encephalitis of unknown origin. All images are at the level of the septum telencephali. Left is on the right of the images. (A) T2-weighted, (B) Susceptibility-weighted, (C) T1-weighted, (D) T1-weighted post-contrast, (E) photograph of formalin-fixed slab of brain tissue – courtesy of G.C.M. Grinwis, Veterinary Pathology Diagnostic Center, Department of Biomedical Health Sciences, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands.

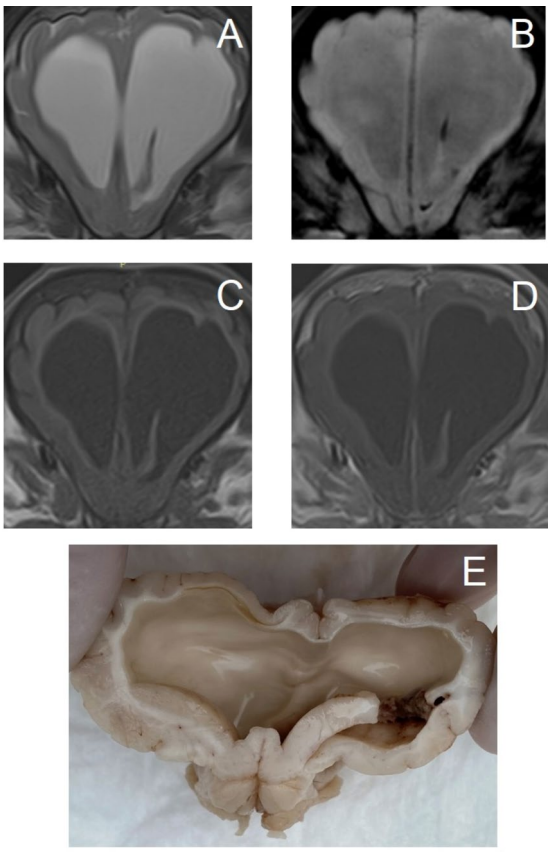

FIGURE 6
Transverse magnetic resonance images and formalin-fixed transverse sectioned brain of a 4-month-old Boxer with a traumatic hemorrhagic encephalopathy – trauma to the head (minor blunt trauma: bumped its head against a wall) without cranial vault fractures, with pre-existing obstructive hydrocephalus due to congenital mesencephalic aqueduct stenosis. (A–D) images are at the level of the septum telencephalic. Left is on the right of the images. (A) T2-weighted, (B) Susceptibility-weighted, (C) T1-weighted, (D) T1-weighted post-contrast, (E) caudorostral mirrored photograph of formalin-fixed sectioned brain at the level of the head of the caudate nuclei (the hemorrhage is on the left side, mirror-image for comparison with MRI).

- *In vivo* biopsy and histopathology and/or post-mortem examination. It must be noted that histopathological confirmation of a diagnosis is almost always required to obtain certainty about the diagnosis and thus the classification of hemorrhagic encephalopathies.

Treatment considerations

As mentioned for diagnostic considerations, the possibility of trauma should be taken into consideration for treatment decisions. In cases of traumatic brain injury and hemorrhage, a peracute or acute and progressive onset of signs is expected and rapid treatment to preserve adequate cerebral perfusion and oxygenation is vital. Briefly, oxygen supplementation and fluid therapy are the pillars of achieving these goals. Aside from maintaining intravascular volume and blood pressure, decreasing intracranial pressure by means of infusion therapy (e.g., mannitol, hypertonic saline) or surgery (e.g., emergency craniotomy) are major considerations in the acute phase (22). Treatment for traumatic brain injury is reviewed extensively elsewhere and there is no current evidence for specific hemorrhage-focussed therapy in veterinary medicine (27).

For the treatment of primary hemorrhagic encephalopathy, aside from that associated with hypertension, no underlying causes can be specifically addressed and treatment focuses on the same principles as treatment for traumatic brain injury: preserve adequate cerebral perfusion and oxygenation. Any other complicating factors, such as epileptic seizures or metabolic derangements, should be adequately and swiftly treated. The main focus is thus to 'buy time' for the intracranial environment to achieve homeostasis and for the brain (as an organ) to slowly repair the damage and degrade the hematoma (s).

In those hemorrhagic encephalopathies where intracranial pressure is elevated acutely, and medical treatment does not result in adequate stabilization, consideration should be given to the surgical approach for potential evacuation of a hematoma. The goal remains the same, which is to achieve adequate cerebral perfusion and oxygenation, and thus prevent further damage and provide the

TABLE 3 Veterinary classification scheme for hemorrhagic myelopathies as suggested by the authors.

Category	Subcategory	Examples
Traumatic spinal hemorrhage	Exogenous trauma	Automobile related, Falls, Kicks etc.
	Endogenous trauma	Disk Extrusion – compressive, non-compressive and intradural/ intramedullary
		Atlanto-axial subluxation
		Iatrogenic
Non-traumatic spinal hemorrhage	Idiopathic	Primary hematomyelia
	Primary hemostatic disorders	Hemophilia
		Immune-mediated thrombocytopenia
	Secondary hemostatic disorders	*Angiostrongylus vasorum*
		Rodenticide poisoning
		Snake envenomation
	Vasculopathy	Vascular malformations
		Vasculitis Primary e.g.,Steroid-responsive meningitis-arteritis (SRMA) Secondary, e.g., Infectious meningomyelitis - *Leishmania*
		Hemorrhagic transformation of an ischemic lesion (e.g., fibrocartilaginous myelopathy)
		Radiation therapy
	Neoplasia	Hemangioblastoma, astrocytoma, ependymoma and metastatic hemangiosarcoma

opportunity of restoring brain function. For non-traumatic hemorrhagic (intraparenchymal) hemorrhage, there is currently no evidence-based indication for surgical treatment in veterinary medicine. Indication for surgical treatment should thus be based on best clinical judgment by the primary clinicians.

The treatment of secondary hemorrhagic encephalopathy also includes the above but will be supplemented with treatment for the specific underlying cause, if such treatment is possible. For instance, bacterial encephalitis will require treatment with antibiotics, and rodenticide poisoning (depending on the type) can be treated with vitamin K. In short, when underlying causes are identified, the treatment is aimed at addressing this underlying cause. For many of the possible etiologies, however, this may not be possible short-term (e.g., neoplasia) or not possible at all (degenerative disorders).

While treatment options for clinical veterinary patients are fairly limited, treatment considerations for human counterparts are reviewed and discussed extensively in the literature (25, 28, 29). Many of the specific treatment considerations for humans are not available or feasible (due to timing) for veterinary patients, while those that are lack firm evidence to support their routine implementation. Human hospitals frequently have dedicated stroke care units – a fact that underlines the importance of timing and protocols in the management of hemorrhagic stroke. Medical treatments include those focused on lowering blood pressure in cases of hypertension to stop active bleeding or reduce the odds of further bleeding, use of hemostatic agents, and anticoagulant reversal agents. Surgical treatment of intracranial hemorrhage in people is supported by evidence while some aspects do remain controversial, such as the exact timing of the surgery. Nonetheless, surgery within a matter of hours (<8 h) has been associated with improved outcomes (25, 28, 29).

Prognostic considerations

In every peracute or acute presentation related to hemorrhagic encephalopathies (whether known immediately or evidenced by further tests later on), the modified Glasgow Coma Scale (MGCS) can be considered to be useful, at least in dogs (30, 31). The ability to assess numerically for deterioration or improvement longitudinally provides the clinician with a valuable tool in decision-making and owner communication. However, it must be kept in mind that, in essence, any such tool relies heavily on the most basic neurological clinical tests, and therefore repeated general physical and neurological examinations are the foundation of assessments of the effectiveness of initiated treatment and expected development.

There are no specific guidelines for prognostication of hemorrhagic encephalopathies in clinical veterinary patients in general, as there are many possible etiologies. For primary hemorrhagic encephalopathies, medium-and long-term prognosis may be very good when the animal survives the short-term as most hemorrhages will abate and resultant hematomas will be cleared eventually. Long-term sequelae (e.g., epilepsy) might negatively influence long-term outcomes or survival (e.g., if owners opt for euthanasia due to such complications), but has not been studied. If primary hemorrhagic encephalopathy is due to hypertension, the ability to treat for hypertension and any underlying disorders itself will influence the prognosis. For secondary hemorrhagic encephalopathies, prognosis will highly depend on the underlying cause. The presence of hemorrhage associated with neoplastic disorders has a very different prognosis from hemorrhage secondary to hemorrhagic diathesis related to *Angiostrongylus vasorum* infections, for instance (being generally worse for the former and good for the latter with short-term survival and appropriate treatment) (32–34).

From literature documenting numerous methods that can be of use to prognosticate patients with head trauma (24, 30, 31, 35–37), it is clear that even the most severely affected patients can show significant short-and long-term improvement. It is prudent to be cautious at early stages of presentation of any patient presented for traumatic hemorrhagic encephalopathy based on these observations. Specific prognostic factors and schemes that have been reported in the veterinary literature include MRI and CT grading schemes (e.g., presence of fractures, mass effect, herniation, compartmental involvement), Modified Glasgow Coma Scale and other clinical grading schemes, and point-of-care tests, such as blood glucose levels in dogs (hyperglycemia negatively associated with survival). However, these pertain to head trauma, not specifically those with or without hemorrhage, and not at all to non-traumatic hemorrhagic encephalopathies.

For humans, specific factors associated with outcome have been reported in the literature. These include the presence or absence of hypertension, localization and extension of hemorrhage, age, pathogenesis (i.e., underlying causes), functional and

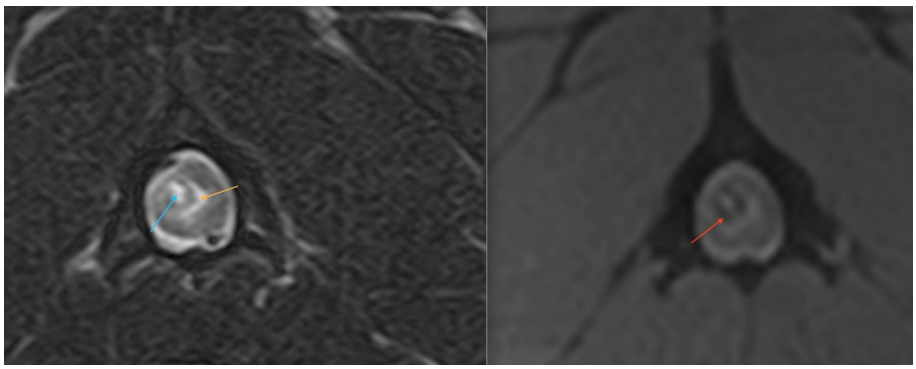

FIGURE 7
A transverse T2W (right side) and T2* (left side) MR image at the level of mid-body C2 of a 2-year-old French bulldog that had been hit by a car and presented with acute onset non-ambulatory tetraparesis. There were no evident osseous lesions noted on cervical vertebral radiographs. There is a relatively well-defined, ovoid intramedullary lesion within the right dorsal quadrant of the spinal cord which has a T2W and T2* hyperintense core (blue arrow), a hypointense rim (red arrow) and peripheral hyperintensity compatible with perilesional edema (orange). The lesion is compatible with a peracute traumatic intramedullary hematoma (49).

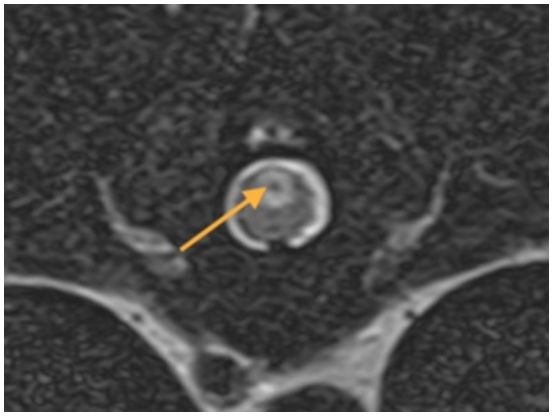

FIGURE 8
A transverse T2W MR image at T10-T11 of a 4-year-old Staffordshire Bull terrier with acute onset a traumatic non-compressive disk extrusion at T12-T13 and paraplegia. There is a well-defined ovoid dorsal midline intramedullary lesion with a T2W hypointense core (arrow) suggestive of hemorrhagic-necrotic material associated with the central canal. The MRI study was compatible with ascending hemorrhagic myelomalacia.

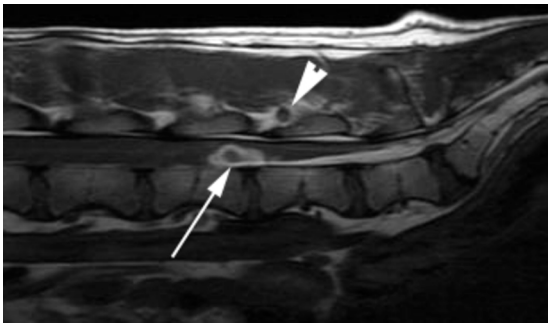

FIGURE 9
A sagittal T1W image of the caudal lumbar vertebral column of a 2 year-old Hungarian Vizsla 4 days after lumbar CSF acquisition and the subsequent onset of a flaccid paraparesis and tail paresis. There is a well-defined ovoid ventral intramedullary lesion centered over the L5-L6 intervertebral disk space (arrow). The lesion has a hypointense core and an irregular hyperintense periphery. A similar lesion is seen in soft tissues dorsal to the interarcuate space at this site (arrowhead).

imaging-based scoring schemes, timing of treatment including surgery, *et cetera* (25, 28, 29, 38–41). Future studies in clinical veterinary patients may yield more useful prognostic indicators for clinicians to consider.

Hemorrhagic myelopathies

Etiological classification

Human

Intraspinal hemorrhage is a rare clinical entity in humans. In Jellinger's classification, human hematomyelia (intramedullary hemorrhage) is divided into three etiological groups: traumatic, secondary, and idiopathic (42).

Trauma is usually associated with vertebral fractures and or luxations which are often concurrently easily visible on imaging studies. However, hematomyelia can be associated with spinal cord injury (SCI) without radiographic abnormalities (SCIWORA) (43). SCIWORA is a term that denotes objective clinical signs of posttraumatic spinal cord injury without evidence of fracture or malalignment on plain radiographs and computed tomography (CT) of the spine. SCIWORA is most commonly seen in children with a predilection for the cervical spinal cord due to the increased mobility of the cervical spine, the inherent ligamentous laxity, and the large head-to-body ratio during childhood (43). Since this is a fairly rare clinical entity, much of its exact pathophysiology remains unknown but it has been suggested that the shearing and stretching of the spinal cord caused by its motion relative to the bony vertebrae at impact might lead to the rupture of intraparenchymal vessels (44).

Up to one-third of non-traumatic cases are idiopathic and these cases are often termed spontaneous hematomyelia (45).

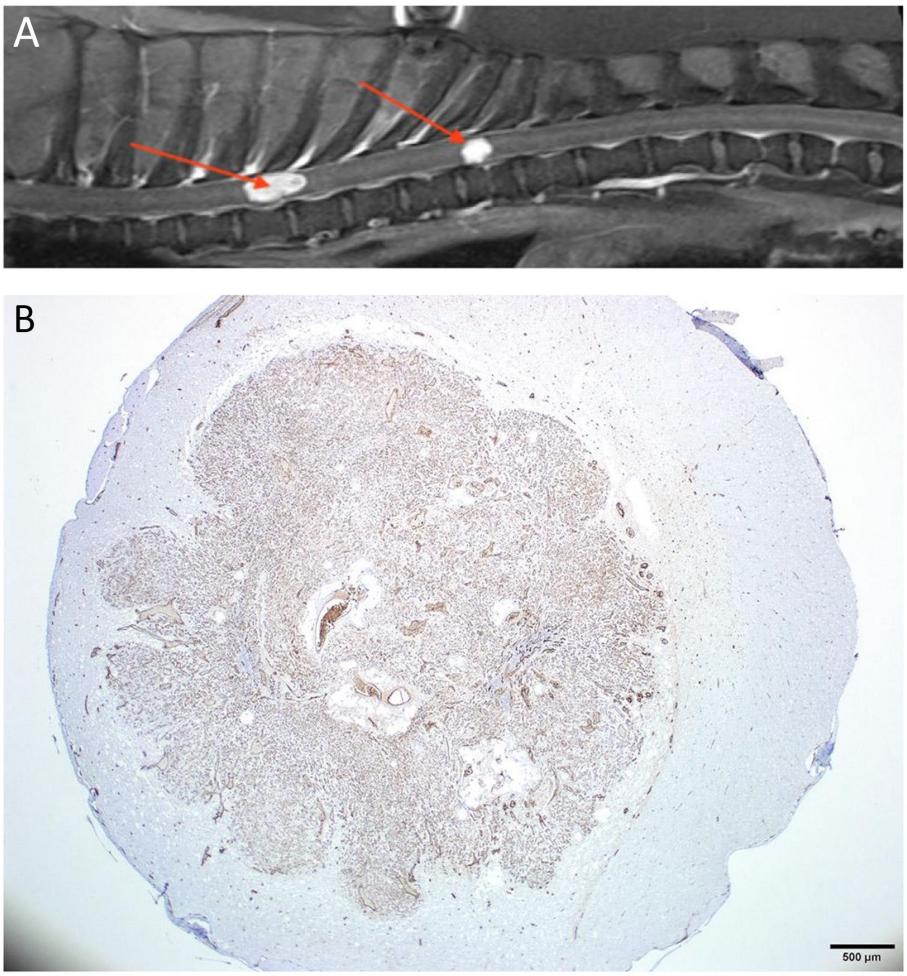

FIGURE 10
(A) Sagittal T1W post-contrast thoracolumbar vertebral column MRI of a 5-year-old mixed-breed dog with a chronic progression of paraparesis and back pain. There are 2 well-defined markedly contrast-enhancing intramedullary lesions (arrows). (B) A histopathological transverse section of the spinal cord at the level of the caudal lesion seen in A. The section is stained for the expression of factor VIII/von Willebrand factor (FVIII/vWF), a specific marker for endothelial cells and reveals that the intramedullary lesion intensely expressed the stain. The lesion was compatible with a hemangioblastoma.

Secondary hematomyelia is mostly associated with spinal cord-specific disorders. Most commonly this has been associated with anticoagulant treatment such as warfarin or heparin (45, 46), but may also be the result of tumors, radiation therapy, or vascular malformations (47, 48). A rare cause of bleeding within the spinal cord is Gowers' intra-syringal hemorrhage, which occurs from a sudden increase of pressure within the syringomyelia cavity and secondary vessel rupture (46).

Veterinary literature and classification proposal

Based on the veterinary literature, spinal cord hemorrhage can be initially divided into traumatic and non-traumatic causes (Table 3).

A) Traumatic spinal hemorrhage (TSH) is suggested to be more common than non-traumatic causes and can include exogenous trauma such as vehicle-related accidents, falls and kicks, endogenous trauma which is related to disk extrusions, and iatrogenic trauma, e.g., after lumbar cerebrospinal fluid [CSF] acquisition.

a Exogenous trauma

As for humans, this type of trauma is usually associated with vertebral fractures and or luxations easily visible on imaging studies. SCIWORA has been reported in veterinary medicine with two dogs and a cat documented to experience hematomyelia secondary to a road traffic accident and falls from a height; in all cases, radiography and MRI revealed no fracture or subluxation (44, 49) (Figure 7). Additionally, cervical hematomyelia has been reported in a cat secondary to a suspected bite injury to the neck, without imaging evidence of a fracture or luxation (50).

b Endogenous trauma

i Intervertebral disk extrusion (IVDE)

Although hemorrhage is a consequence of acute disk extrusion, whether it be compressive, non-compressive nucleus pulposus extrusion or intradural/intramedullary disk extrusion, it may also be a contributing factor to the further progression of traumatic myelomalacia, a significant structural disruption of the spinal cord

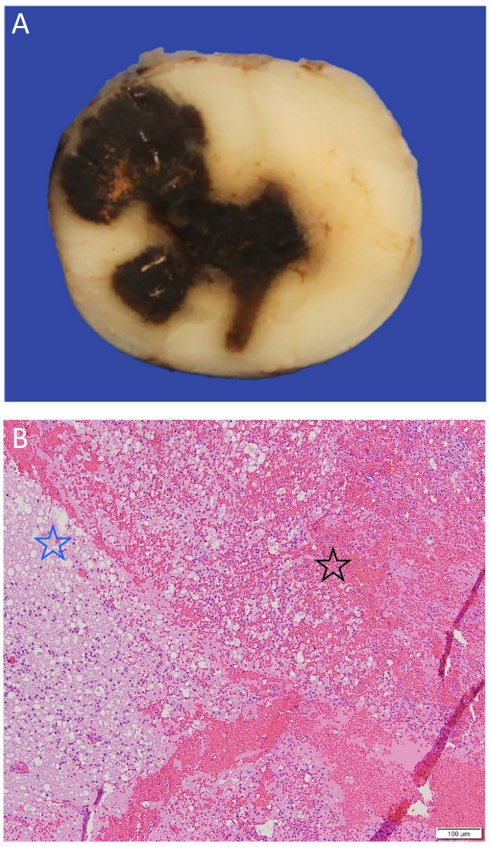

FIGURE 11
(A) A gross pathological cross-section of the cranial thoracic spinal cord of a 4-year-old French bulldog with acute progressive paraparesis secondary to primary hematomyelia. There is an extensive drak red/black region of hemorrhage seen replacing the neuroparenchyma. (B) An H&E x 5 image of the border of the extensive hemorrhage (black star) seen in A, which can be seen to be effacing and replacing the neuroparenchyma and compressing the adjacent spinal cord tissue (blue star).

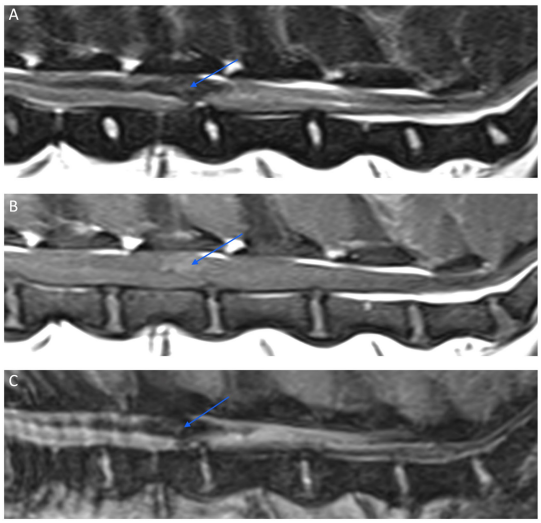

FIGURE 12
Sagittal MR images of the caudal lumbar vertebral column of a 9-year-old Beagle with suspected primary intramedullary hemorrhage. A relatively well-defined irregular intramedullary hypointense lesion (arrow) is seen on a T2W image (A) extending over the L3 and L4 vertebral bodies. The lesion has a mild T1W pre-contrast hyperintense signal (B), and exhibits a signal void on a Multiple Echo Data Image Combination (MEDIC) (C), which is a T2*-weighted spoiled gradient echo sequence.

secondary to intervertebral disk extrusion (51–54). Experimental studies suggest that the mechanism of this contribution involves detrimental biochemical effects of blood products on spinal cord tissue (55). The extent of intramedullary hemorrhage is significantly associated with the severity of spinal cord destruction at the site of the disk extrusion (56). In severe canine SCI following IVDE, blood can enter the central canal, presumably after its mechanical disruption at the epicenter of the lesion, which frequently leads to massive distention of the central canal in adjacent and remote segments (Figure 8). This subsequently leads to the rupture of the central canal with extrusion of the hemorrhagic debris into the dorsal column area suggesting the presence of a driving force propelling this material rather than the spread of the hemorrhagic debris by passive leakage.

ii Developmental anomalies

Similar to IVDE, the most common cervical vertebral developmental anomaly, atlanto-axial subluxation, can be responsible for traumatic damage to the overlying cord and in some cases is associated with hematomyelia, which can complicate prognosis (57).

iii Iatrogenic

Accidental or unintentional intraspinal hemorrhage unassociated with surgery has been reported with myelographic procedures, cerebrospinal fluid acquisition in addition to inadvertent thoracic intraspinal injection (58, 59). The origin of the hemorrhage in both circumstances is purported to be the puncture of a parenchymal vessel which results in a sudden onset of clinical signs within hours of the lumbar puncture (Figure 9). However, although computed tomographic examination of the spinal cord of dogs after lumbar myelography has confirmed the presence of intramedullary hemorrhage, it is not always associated with clinical signs (60).

B) Non-traumatic spinal hemorrhage (NTSH) can be idiopathic or secondary to an underlying disease process or medical condition (61). Several medical conditions have been associated with NTSH in dogs, many of which are associated with an effect on the coagulation capabilities of the animal.

a Primary hemostatic disorders have been associated with:

i Hemophilia

Hemophilia is the most common congenital coagulation disorder affecting secondary hemostasis in humans (62). Hemophilia A is an X-linked deficiency in factor VIII and hemophilia B is caused by a deficiency in factor IX (63). Hematomyelia and hematorrhachis have been reported in juvenile dogs with hemophilia A and a cat with hemophilia B (64–67). CNS bleeds in humans with hemophilia has a prevalence of approximately 7.5% with no history of trauma in 67% of these cases; only 1% of these hemorrhages were intraspinal (68).

ii Immune-mediated thrombocytopenia

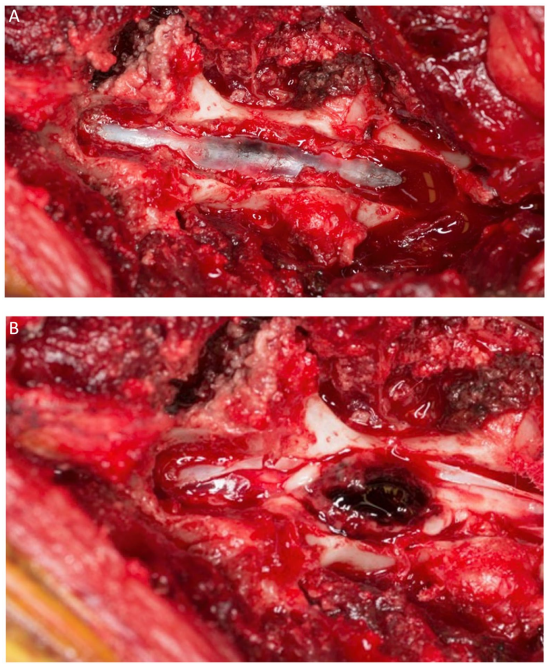

FIGURE 13
A dorsal view of the spinal cord of the dog of Figure 12 after a dorsal laminectomy (A) and a durotomy (B) revealing a hematoma on the dorsal midline of the cord just prior to its removal.

A 4-year-old neutered female Bearded Collie has been reported with progressive tetraparesis, immune-mediated thrombocytopenia and presumed secondary spinal cord hemorrhage (69).

b Secondary hemostatic disorders have been reported in association with:

i *Angiostrongylus vasorum* (*A. vasorum*)

Common in Europe but rare in the United States, *A. vasorum* is a metastrongylid nematode worm that infects dogs after ingesting snails or slugs. This often results in a secondary coagulopathy and pulmonary hemorrhage, but central nervous system hemorrhage has been reported (70). Thrombocytopenia, anemia and eosinophilia are frequently observed in infected dogs, and diagnosis is usually based on finding the larvae in feces (71). Anthelmintic therapy and supportive care are recommended but anaphylactic reactions have been suspected, triggered by the rapid release of a large amount of worm antigen into the blood as the worms were killed (70).

ii Rodenticide toxicity

Warfarin competitively inhibits the vitamin K epoxide reductase complex 1, which is essential for activating vitamin K. Vitamin K is an essential cofactor for the hepatic synthesis of multiple clotting factors including II, VII, IX, and X and factors protein C and protein S. As such, the main adverse effect of warfarin is hemorrhage, which is not predictable based on the dosage administered. Anticoagulant rodenticides initially cause prolongation of the prothrombin time at 24–36 h post-ingestion. This coincides with a depletion of Factor VII which has the shortest half-life of all the vitamin K-dependent factors (6.2 h in the dog) and whose activity is measured by the PT. (72) Prolongation of the aPTT follows when there is a depletion of other coagulation factors. A 3-year-old Boxer dog has been reported with hematomyelia secondary to warfarin ingestion causing non-ambulatory tetraparesis (73). Extradural hemorrhage has also been reported in a dog exposed to diphacinone (74).

iii Snake envenomation

A dog has been described with coagulopathy secondary to a brown snake (Pseudonaja species) which experienced paraparesis within 12 h and which progressed over the next 2 days (75). An extradural hematoma was removed surgically and the dog recovered with minimal complications. Although cerebral hemorrhage has been described, secondary to snake bites, the authors could not find evidence of human or veterinary cases of hematomyelia.

c Vasculopathy - there are multiple non-traumatic conditions that affect the structural integrity of the vascular supply to the cord:

i Vascular malformations

CNS vascular lesions have been categorized into three major groups: (i) reactive vascular proliferations, (ii) vascular malformations including benign neoplasms (e.g., hemangioma) and hamartomas, and (iii) neoplastic disorders (e.g., hemangioblastoma, hemangiosarcoma) (13). The most widely accepted classification system of CNS vascular malformations in people separates lesions into arteriovenous malformations (AVMs), cavernous malformations (CVMs), venous malformations, and capillary telangiectasia (76); a similar classification appears to exist in veterinary medicine (77). Venous malformations and capillary telangiectasia do not carry a significant risk for acute hemorrhage, unlike AVMs and CVMs. Spinal cord AVMs in people can be further divided into dural arteriovenous fistulae and intradural AVMs, which include glomus, juvenile, and fistulous subtypes (78).

Very few reports of vascular malformations exist in veterinary medicine but there have been descriptions of associated hemorrhage causing clinical signs, their imaging characteristics and surgical resection (79–87). Specifically, intramedullary cavernous malformations were described in 2 dogs with myelography identifying an intramedullary lesion proven to be extensive intraparenchymal hemorrhage on postmortem associated with a distinct lobulated intramedullary mass (79); 2 other dogs with intramedullary hemangiomas have had their MRI characteristics described confirming hyperintensity on both T1W and T2W sequences (82); an intramedullary hemangioma was excised via a myelotomy in a 5 ½-year-old Leonberger and an undefined vascular malformation was removed similarly from a 3 ½-year-old cross-breed dog and a 1-year-old Labrador retriever, with long term remission achieved in all 3 (80, 83, 84);

ii Vasculitis

Vasculitis refers to inflammation of the blood vessel wall that may develop without apparent cause (primary vasculitis), or in response to a range of initiating insults (secondary vasculitis) (88). There are few defined syndromes in veterinary medicine that are referable to primary vasculitis, but these include the systemic necrotizing polyarteritis syndrome described in colonies of laboratory Beagle dogs, now identified clinically in many breeds and referred to as steroid-responsive meningitis-arteritis (SRMA) (89). SRMA typically results in

juvenile dogs experiencing severe neck pain, fever, and an inflammatory leukogram. Dogs with SRMA can experience secondary extradural, intradural-extramedullary or intramedullary hemorrhage (90–93). Spinal hemorrhage has been documented to affect 9.4% of 53 dogs with SRMA and was associated with the presence of paresis/paralysis (93).

Secondary vasculitis has been reported in association with a wide range of infectious, inflammatory, and immune-mediated diseases in animals and humans. Of these, reported causes include autoimmune diseases such as systemic lupus erythematosus (SLE), infection with *Leishmania* spp., *Rickettsia* spp., *Angiostrongylus vasorum*, and *Dirofilaria immitis*, and exposure to therapeutic products such as carprofen and meloxicam (88).

In canine leishmaniasis, immune complexes may be deposited on blood vessels as a consequence of the persistent production of circulating antigens. These soluble complexes activate the complement cascade, which elicits an inflammatory response and may cause systemic (necrotizing) vasculitis (94). A 14-month-old cross-breed dog with paraplegia has been reported with hematomyelia secondary to leishmania and vasculitis (95).

iii Hemorrhagic transformation of an ischemic lesion (e.g., fibrocartilaginous myelopathy)

Infarcted areas of the spinal cord secondary to fibrocartilaginous embolic myelopathy are usually ischemic but there can also be a hemorrhagic component (61, 96).

iv Radiation therapy

Hematomyelia has been reported secondary to spinal cord radiation therapy (61, 97). Hemorrhage primarily involved the white matter, but also involved one or more of the gray matter horns at different levels of the spinal cord. The ED_{50} for massive cord hemorrhage is 65.8 Gy based on one histopathologic study (97).

d Neoplastic disease

Hemangioblastoma, astrocytomas, or ependymomas may have intralesional hemorrhage. However, they normally show a solid portion and some degree of contrast enhancement (Figure 10) (98). Extradural hemorrhage has been associated with a primary epidural hemangiosarcoma in a dog (99). The MRI features of both extra-and intramedullary hemangiosarcomas have recently been described, with intramedullary lesions being cervical, metastatic, and frequently accompanied by intracranial lesions (100).

e Idiopathic

When no cause of the hemorrhage can be identified, the etiology is classified as idiopathic which is the most common single classification when considering NTSH (35% of 23 dogs) (61). This has been termed primary hematomyelia (101, 102) (Figure 11).

Localization

Regardless of species, hemorrhagic myelopathies can be classified based on the location of the bleeding just as they can for encephalopathies, even though this review focuses on intramedullary bleeds. Intraspinal but extramedullary hemorrhage is termed hematorrhachis while hemorrhage within the spinal cord is called hematomyelia. Spinal hematoma location(s) include epidural, subdural, subarachnoid, intramedullary, or a combination of these. This may be further classified based on specific localization along the craniocaudal axis, left–right lateralization, and gray versus white matter, for instance.

In humans, subarachnoid hemorrhage in the spine occurs in 6/100,000 people annually. Approximately 85% of the time, SAH in the spinal column is related to an intracranial aneurysm rupture (45) Spinal epidural hematoma is the next most common spinal hemorrhage in people, although still rare. The incidence of this pathology is approximately 0.1 per 100,000. Spinal EDH is usually caused by epidural venous plexus hemorrhage, which may be caused by minor trauma, coagulopathy, transmitted venous hypertension, or intervertebral disk herniation (103).

In veterinary medicine, although specific localization is difficult to confirm based on imaging studies, spinal hemorrhage may be located in any of the following locations (61):

- Intradural-extramedullary
  - o Subarachnoid hemorrhage
  - o Subdural hemorrhage
  - o Subpial hemorrhage
- Intramedullary
- Extradural

Further anatomical differentiation of intramedullary hemorrhage may focus on segmental localization (i.e., C1-C5, T3-L3, etc.) and whether it can be determined to be affecting white matter, gray matter, or the central canal.

Clinical signs

Clinical signs related to hemorrhagic myelopathies are primarily dependent on the location of the hemorrhage within the spinal cord tissue, extent of hemorrhage, associated pathology, secondary effects (e.g., mass effect), and time frame (e.g., acute versus chronic phase). Therefore, signs vary, but predominantly include paresis (lower motor neuron when cervical or lumbosacral intumescences are involved), proprioceptive ataxia, spinal reflex deficits, proprioceptive deficits, abnormal postures, hyperesthesia, and abnormal sensation.

The majority of cases of NTSH will have an onset of clinical signs within 72 h and up to 70% can exhibit progressive neurological signs from hours to several weeks (61); however, the median duration of progression is 24 h. Several grading systems have been used to assess the severity of neurological signs in dogs and cats with a spinal cord lesion. These include the Texas Spinal Cord Injury Score and the Modified Frankel Scale (104, 105).

For humans patients with hematomyelia, a clinical triad of local spinal pain, radicular pain, and long-tract signs has been described (106).

Diagnostic considerations

In cases with suspected TSH, a systemic physical and imaging evaluation should be considered before further evaluation of the vertebral column, with an urgent focus on the evaluation of the

patient's airway, breathing function, circulation, and the possibility of ongoing exsanguination. Imaging assessment of the vertebral column should preferably be performed using CT and or MRI and should not solely focus on the lesion localization determined by a neurological examination in case of multifocal injury.

The most sensitive diagnostic test to confirm the presence of intramedullary hemorrhage is MRI. Differentiating spinal hematoma from inflammatory or neoplastic lesions can be challenging. The clinical history and contrast-enhanced imaging may help to differentiate pathologies, i.e., malignancy such as infiltrative lymphoma or metastasis will enhance although contrast enhancement of intraspinal hemorrhage has been reported in dogs (66, 107). Contrast imaging may identify underlying intramedullary lesions such as hemorrhagic ependymoma which enhance avidly. Additionally, intramedullary hemorrhage is frequently accompanied by cord edema, seen as hyperintensity on T2 weighted sequences, with loss of the gray-white matter differentiation on transverse images. MR imaging has been described in experimental dogs with acute traumatic lesions of various severities which found variation in location and extent of the resultant hemorrhage based on the associated severity of the impact (108). In severe traumatic-impact injuries. MR showed widespread longitudinal extension of the hemorrhage with involvement of the central and periphery of the spinal cord versus a more central location after low-impact injuries.

As for intracranial hemorrhage, the imaging features of hematomas on MRI evolve over time (109); the progression of hemoglobin degradation and therefore the imaging findings may proceed differently than in the brain due to variability in the local environment, but this has not been established in the dog (107). Due to the presence of intracellular oxyhemoglobin, spinal hematoma in the hyperacute phase appear isointense on T1 and hyperintense on T2 weighted imaging. A rim of hypointensity surrounding the hematoma is variably seen. Intracellular deoxyhemoglobin accounts for acute phase imaging characteristics; T2 signal intensity decreases and hematoma appears hypointense. T1 signal remains intermediate to long and hematoma, therefore appears hypo−/isointense. In the early subacute phase, T1 signal intensity increases, T2 shortens and hematoma appears T1 hyperintense and T2 hypointense (Figure 12). In the late subacute phase, extracellular methemoglobin is formed and hematoma is visualized as both T1 and T2 hyperintense. Paramagnetic hemosiderin and ferritin account for the usual appearance of chronic hematoma which is hypointense on both T1 and T2 weighted imaging. It should be noted that signal voids on spin-echo sequences are associated with gas, cortical bone, calcification, fibrous tissue, metallic implants, fast-flowing blood, and blood-breakdown products (110).

A gradient echo T2-weighted (GRE) sequence is the most sensitive method to detect hemorrhage (111). However, a signal void on GRE sequences may not be seen in hyperacute stages (before conversion to deoxyhemoglobin) or chronic hemorrhage (because of the homogenous distribution of extracellular methemoglobin) (112).

CT of the spine may also be used to identify the hemorrhage and any evidence of associated spine pathology. Spinal cord hemorrhage can present as an expansile short or long segment area of hyperattenuation on CT images, which may be multifocal and may be contrast-enhancing potentially surrounded by a hypoattenuating region of edema (45, 64). However, overall MRI is a more valuable technique for the detection of chronic hemorrhage, which may be invisible on CT (113).

Specific etiology testing should focus on the potential for a bleeding diathesis starting with evaluation for *A. vasorum* using an in-clinic assay for detection of circulating antigen (Angio Detect Test, IDEXX Laboratories, Westbrook, Maine, USA) in addition to looking for the presence of first-stage *A. vasorum* larvae on fecal analysis (Baermann method). Further testing should include coagulation assays [prothrombin time (PT), activated partial thromboplastin time (aPTT), buccal mucosal bleeding time (BMBT)] and if there are abnormalities, they should be followed by platelet quantification and specific coagulation factor testing.

In humans, in addition to standard MR or CT imaging, magnetic resonance angiography is often considered and CT angiography is a valid alternative (46). Angiography involves sequential, multiple injections of potentially parent vessels to identify the vascular pathology. However, locating the source of the hemorrhage by angiography is often a challenge especially when an isolated aneurysm is the culprit. MR and CT spinal angiography has been described and used to evaluate the vasculature of the canine spinal cord (114, 115).

Treatment considerations

In the absence of clinical trials to guide the treatment of these rare conditions, management is often aimed at underlying causes if known, and supportive care. Conservative treatment may be justified in cases with minimal or rapidly improving neurological deficits and has been documented to be successful in dogs with TSH (49). If TSH is suspected, basic support measures and general patient stabilization is required before focusing on specific treatment measures in order to maintain adequate spinal cord perfusion (116). Maintaining blood pressure within standard reference ranges can be and is regarded as a cornerstone of treatment for this aim (117).

However, there does not appear to be a consensus on whether medical or surgical treatment is.

more appropriate and it is currently suggested that conservative management may be reserved for cases with mild neurological manifestation or increased bleeding tendency, whereas surgery is a viable option when the neurological signs are severe and progressive, and when removal of the expanding hemorrhagic lesion may be beneficial (44).

In humans, management of the acute case is focused on relieving pressure on the spinal cord where individual reports suggest that surgical decompression should be performed as soon as possible to minimize the neurological injury (46). Durotomy and duraplasty have been studied in humans with SCIWORA as a mechanism to indirectly relieve intraspinal pressure (118). Myelotomy has also been described in animal models of SCI and is sporadically reported with positive effects in humans (119). The specific surgical management of intramedullary hemorrhage focuses on evacuation of the mass effect and parenchymal decompression, minimizing disruption of normal spinal cord parenchyma (Figure 13). Intramedullary hemorrhage, which extends to the surface of the pial spinal cord may establish a safe microsurgical corridor of entry. Successful decompressive surgery of an intramedullary hematoma via myelotomies has previously been described in several dogs and a cat with various underlying etiologies (44, 58, 77, 101).

Prognostic considerations

In veterinary medicine, outcomes with NTSH have been investigated relative to the underlying cause, MRI findings, lesion localization, and severity of neurological signs. No association was found between outcome and the presence of an underlying cause when grouping dogs with underlying causes of NTSH together and comparing to the idiopathic group in a study of 23 dogs (61). However, underlying causes associated with a poor outcome include radiotherapy-induced hemorrhage, acute lymphoid leukemia, FCE, and hemophilia A.

In relation to MRI findings, a study of 23 dogs with NTSH found that the mean length of the hemorrhagic lesion was 4 times higher and the mean length of spinal cord edema was 2 times higher in dogs with a poor outcome compared to dogs with an excellent outcome (61). However, these findings were not significant. Based on this same small study, and unusually, no significant association was found between outcome and severity of neurological signs at initial presentation. A study of 82 dogs presenting with paraplegia and absent nociception secondary to disk extrusion demonstrated that only 33% regained nociception if MRI revealed intramedullary gradient echo signal voids, compared to 67% that recovered and did not have such voids (120).

Outcome associated with TSH is related to clinical signs at the outset. For dogs with disk extrusion, the extent of intramedullary hemorrhage is significantly associated with the severity of spinal cord destruction at the site of the herniation (56). Additionally, the degree of intramedullary hemorrhage is significantly associated with the rostrocaudal extension of myelomalacia suggesting that hematomyelia may contribute to the further progression of myelomalacia. Hemorrhage within the central canal in spinal segments cranial and caudal to the disk extrusion is a finding strongly associated with high grades of tissue destruction and in one study was seen in 75% of dogs with ascending-descending myelomalacia (56).

In human patients with hemorrhagic myelopathy, no association has been found between patient outcome and the location or extent of the hemorrhagic lesion on MRI. However, patients with more severe neurological signs, such as complete loss of motor function, loss of nociception, urinary or fecal incontinence, or both, have been reported to have a significantly worse outcome (121).

Concluding remarks

As hemorrhagic encephalopathies and hematomyelia are more frequently diagnosed in veterinary patients through advanced imaging studies correlated to clinical presentation, classification according to etiology becomes more important to investigate whether certain categories are associated with poor or good outcomes, require surgical intervention, or have implications for diagnostic procedures. Classification systems have been employed for human hemorrhagic encephalopathies and myelopathies and were discussed in this review as background for the proposal of a veterinary classification. The human literature is much more extensive on this topic and thus forms a substantial part of the reference list for this veterinary review. The proposed classifications in this review can be implemented for clinical use and both retrospective and prospective studies.

Author contributions

KS: Conceptualization, Funding acquisition, Visualization, Writing – original draft, Writing – review & editing. SP: Conceptualization, Visualization, Writing – original draft, Writing – review & editing.

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Conflict of interest

SP was employed by Vet Oracle Teleradiology.

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Comparative analysis of chronic neuropathic pain and pain assessment in companion animals and humans

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Chronic neuropathic pain is underdiagnosed in companion animals. This paper will review the definition of pain and how classification and grading of neuropathic pain can be applied from human to veterinary medicine to increase the recognition of and the confidence in a neuropathic pain diagnosis. The mechanisms of nociception and the pathophysiology of the sensory systems that underlie the transition to chronic pain are described. Potential future methods for diagnosis and treatment of neuropathic pain in veterinary medicine are considered, utilizing the theoretical framework of pain behavior from humans and rodents. By discussing the current state of pain diagnosis in companion animals and increasing the recognition of chronic neuropathic pain, the goal is to increase understanding of chronic neuropathic pain in daily clinical practice and to aid the development of methods to diagnose and treat neuropathic pain.

KEYWORDS

chronic pain, neuropathic pain, neurology, translational medicine, canine

1 Introduction

Pain diagnosis and management in human medicine is challenging, but in veterinary medicine, additional factors compound this inherent challenge. Definitions of pain used in human medicine can be applied to veterinary medicine (1). The definition of pain from the International Association for the Study of Pain (IASP) is: "the unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage" (2). The IASP recognizes that the lack of verbal description does not remove the possibility that pain is experienced and therefore recognizes that pain occurs in non-human animals (2). In companion animals, veterinarians and veterinary team members detect and measure pain by observation, examination, and obtaining a through history from pet-owners.

Differentiating pain from anxiety, cognitive dysfunction, or other behavioral disorders is an important aspect of diagnosing pain in companion animals (3–5). Once pain is diagnosed, the goal is to determine the source and type of the pain, for example, separating neuropathic and musculoskeletal pain (6). In veterinary species, as in human medicine, there are limited effective treatment options for neuropathic pain (7, 8). New technologies for diagnosis and treatment of pain in veterinary and human medicine are under development. However, large gaps remain in our understanding of pain pathophysiology in all species.

The diagnosis and measurement of chronic pain in clinical companion animal practice can be improved by refining pain classification, quantifying signs of pain, attempting to

separate signs of pain and anxiety, and developing markers of pain that are not based on clinical examination findings (1). Although several types of pain affect both companion animals and humans, we will focus here on neuropathic pain in companion animals, which is likely underdiagnosed and may be better understood by considering neuropathic pain classification and diagnosis in humans (1, 6, 9). This review will discuss the physiology and pathophysiology of chronic neuropathic pain, challenges and opportunities in veterinary practice, and methods to combine current clinical practices in veterinary medicine with advances in human and rodent pain management to better detect and treat pain in our veterinary species.

2 Classification of neuropathic pain in humans and probable sources of neuropathic pain in companion animals

The perception of pain, or nociception, is normally a protective mechanism. However, chronic pain can also be a maladaptive pathologic disorder (10). Chronic pain of neurologic origin is classified as either peripheral or central in humans and then is further characterized by origin and mechanism (Figure 1A) (6). For neuropathic pain, no similar classification has been established in companion animals. By utilizing this classification scheme and applying it to companion animals, we may improve our knowledge of neuropathic pain in these species.

The neuropathic pain grading system captures information about the probability that neuropathic pain is present, using possible, probable, or definite based on the clinical picture (Figure 1B) (11, 12). This type of classification scheme was originally proposed in recognition of the lack of a universal method to diagnose neuropathic pain, which is a continued problem in veterinary and human medicine (12). Applying these grading criteria to companion animals can capture a clinician's level of certainty that neuropathic pain is present, which may guide treatment. Recording neuropathic pain grade may be useful for deciding when to initiate treatment, determining treatment effectiveness, or documenting information for future retrospective studies.

For each of the major neuropathic pain categories that are described in humans, examples of similar companion animal disorders are shown (Figure 1A). One cause of neuropathic pain in companion animals is caudal occipital malformation (CM) with syringomyelia (SM), which is a chronic central neuropathic pain associated with spinal cord injury (4, 7, 8). Another example of neuropathic pain is degenerative lumbosacral stenosis, which is a painful radiculopathy (13, 14). However, some sources of neuropathic pain in companion animals are under-recognized, such as poststroke pain or painful polyneuropathies. Applying the human classification and grading of neuropathic pain to companion animals may improve the recognition, diagnosis, and treatment of these disorders.

3 Physiology of nociception

The pathways for sensing and processing nociceptive information are part of the "pain, touch, and temperature" system that make up the general somatic afferent systems (GSA) (15). Nociceptive stimuli, such as mechanical or thermal stimuli, are encoded by activation of nerve endings, which may be located in the skin, deep tissues, or organs (16). The most common nerve endings to detect nociceptive signals are free nerve endings, though other cells such as epithelial cells and Merkel cells may contribute to the initial encoding step (16). The cell bodies of these pseudounipolar primary sensory neurons form the dorsal root (DRG) and trigeminal ganglia (TG) (Figure 2A). Primary sensory neurons are classified by the type of information they transmit, axon size, myelination, and conduction velocity. Other classification systems have also been proposed based on gene expression patterns or electrophysiologic properties (16, 17).

The axons from the DRG enter the dorsal horn of the spinal cord via the dorsal root and synapse in the superficial layers of the spinal cord (Figure 2A) (15–17). Nociceptive information from the spinal cord is transmitted to reflexive pathways, local processing occurs, and information is transmitted to the brain. The primary nociceptive pathway in humans is referred to as the spinothalamic tract. However, in companion animals, important nociceptive pathways also include the dorsal column postsynaptic pathway, the spinocervicothalamic pathway, and the spinomesencephalic pathway (15). Nociceptive information from the head is primarily encoded by sensory neurons in the trigeminal nerve. Therefore, the majority of sensory information from the head is transmitted in the quintothalamic pathway. These pathways transmit nociceptive information to several locations in the brain. Together, the GSA system in companion animals can be called the spinothalamic system, though that terminology does not fully capture the myriad pathways of nociceptive transmission (Figure 2B) (15).

While much of the nociceptive information is transmitted via the spinothalamic tract to the primary sensory cortex via the thalamus, several other areas of the brain respond to an acute nociceptive stimulus. These include the insular cortex, cingulate cortex, prefrontal cortex, posterior parietal cortex, the secondary somatosensory cortex, the amygdala, hippocampus, and motor cortex (16, 18, 19). Other areas include the cerebellum, medulla, and periaqueductal gray region. Some of these regions, such as the somatosensory cortex, thalamus, and insular cortex, are important for the sensory aspects of pain, while the cingulate cortex, insular cortex, and prefrontal cortex process the affective aspects of pain (18). The regions of the brain that respond to painful stimuli are sometimes collectively referred to as the Pain Matrix. This concept emphasizes the complexity of processing of nociceptive information.

4 Pathophysiology of chronic pain

Alterations in the cellular and network processing of nociception are thought to underly the development of chronic pain. It may take days to weeks for chronic pain to develop, and these alterations in neuronal function often persist even after the originating tissue damage has resolved (11, 20). These maladaptive

A: Proposed Categorization of Neuropathic Pain Subtypes

Category	Human disorder example	Proposed Companion animal example
Chronic Peripheral Neuropathic Pain		
Trigeminal neuralgia	Idiopathic, vascular, tumor	Idiopathic Trigeminal neuritis, tumor
Chronic neuropathic pain after peripheral nerve injury	Trauma	Trauma, tumor, iatrogenic
Painful polyneuropathy	Chemotherapy induced, toxic, metabolic, etc.	Metabolic, toxic, idiopathic
Postherpetic neuralgia	Post-herpes zoster	None defined
Painful radiculopathy	intervertebral disc disease, tumor	Intervertebral disc disease (foraminal), tumor, lumbosacral stenosis
Residual or unclassified peripheral neuropathic pain category	Carpal tunnel syndrome	None defined
Chronic Central Neuropathic Pain		
Chronic central neuropathic pain associated with spinal cord injury	Trauma, intervertebral disc disease	Trauma, intervertebral disc disease, caudal occipital malformation with syringomyelia
Chronic central neuropathic pain associated with brain injury	Trauma	Trauma, Meningoencephalomyelitis of unknown etiology
Chronic Central Post-stroke pain	Cerebrovascular event	Cerebrovascular event
Chronic central neuropathic pain associated with multiple sclerosis	Multiple sclerosis	Meningoencephalomyelitis of unknown etiology

B: Proposed Grading system for Neuropathic pain

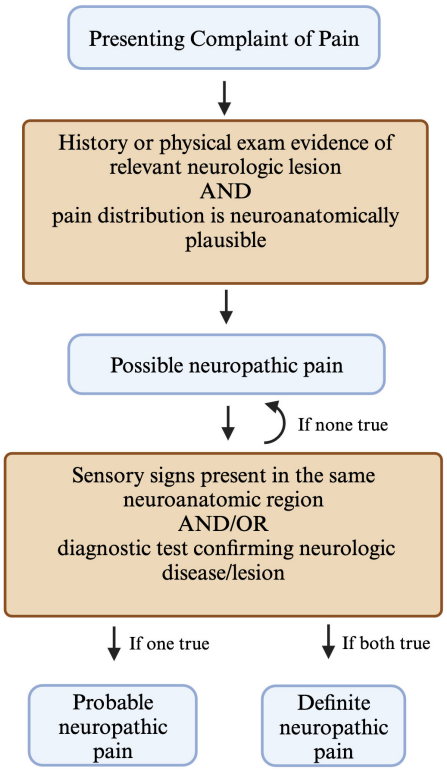

FIGURE 1
(A) Classification of neuropathic pain, including central and peripheral subtypes, with corresponding examples from humans and proposed corresponding neuropathic pain disorders in companion animals. **(B)** Grading system decision tree for confidence in neuropathic pain diagnosis. For patients presenting with pain, a history of relevant neurologic lesions and a matching distribution is consistent with possible neuropathic pain. The presence of either sensory signs (loss of sensation or increased/altered sensation) or lesion confirmation based on diagnostics results in the conclusion of probable or definite neuropathic pain. Created with BioRender.com.

responses occur at several levels in the nociceptive network, including encoding, transmission, and perception (Figure 2).

In chronic pain states, inflammation or injury to the primary sensory neurons results in hyperexcitability and increased firing. Other possible causes of pain may include an imbalance between ascending and descending signaling pathways (20). Chronic pain can result in changes in gene transcription and translation in individual neurons and support/glial cells in the dorsal root ganglia and spinal cord (21). This may cause altered processing of sensory information in the spinal cord. Additionally, changes in the brain's response to painful stimuli also occur, which can be measured as difference in regional blood flow (16, 18, 19). Studies have identified changes in brain volume, including loss of volume in the primary somatosensory cortex and thalamic gray matter or increased tissue volume in the cingulate cortex and primary motor cortex (18, 20).

5 Diagnosis of pain in companion animal clinical practice

The diagnosis of pain for companion animals currently relies on owner reporting, physical examination findings, and direct observation of the pet. The diagnosis of pain is complicated by the inherent limitations of examining animals in the veterinary setting. The stress of a hospital visit may mask subtle aspects of pain the owner may appreciate in a home setting. The role of veterinary visits in anxiety is not well understood (22). The clinician sometimes must rely upon other factors, including patient signalment or owner reports or videos.

Observational findings may suggest the presence of pain, including changes in posture, facial expression, gait, or tone. During the physical and neurologic examinations, other indicators of pain may include heart rate, respiratory rate, or muscle atrophy. Pain or muscle fasciculations may be elicited on palpation. However, not all animals will respond to palpation, and it can be difficult to localize pain. Additionally, in referral settings, many patients have previously received pain medication, which can mask the clinical manifestations of pain.

Structured assessments, such as owner questionnaires for pain, include the Canine Brief Pain Inventory (CBPI), Neuropathic Pain scoring (NeP), and quality of life visual analog scoring (VAS) (4, 23, 24). These are short questionnaires that can be repeatedly filled out by owners over time. However, they are underutilized in practice. Additionally, these forms may rely on animals having a

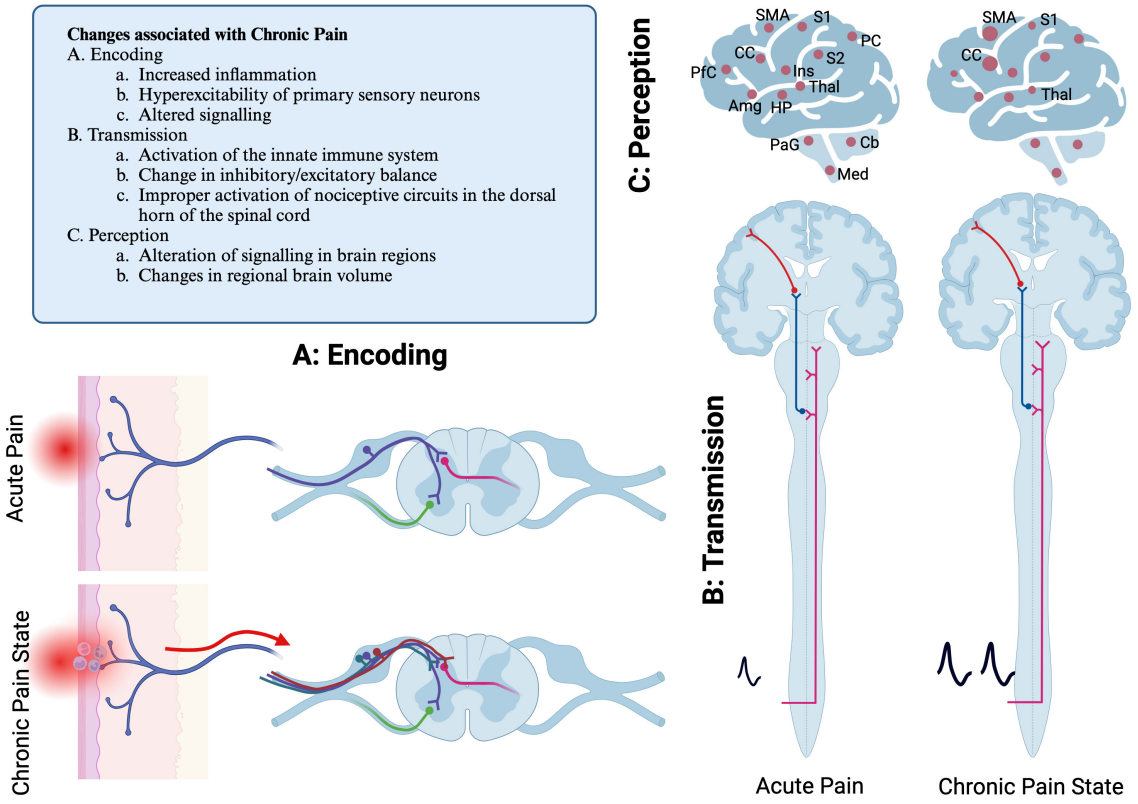

FIGURE 2
Schematic diagram of pain pathways showing primary location of reflexive and ascending pain pathways. Mechanisms of chronic pain development are illustrated as well. **(A)** Encoding step, illustrating a free nerve ending that is activated due to acutely painful stimulus. **(B)** Transmission, the spinothalamic tract is pictured. Not pictured are other sensory pathways, including dorsal column postsynaptic pathway, the spinocervicothalamic pathway, the spinomesencephalic pathway and quintothalamic pathways. **(C)** Illustration of the regions of the brain that are associated with painful stimuli. These may have sensory or affective valence. In chronic pain, regions may either be differentially active, or there can be changes in brain volume. S1, primary sensory cortex; S2, secondary sensory cortex; Thal, thalamus; Ins, insula; PC, parietal cortex; CC, cingulate cortex; PfC, Prefrontal cortex; HP, hippocampus; Amg, amygdala; PaG, periacqueductal gray; Cb, Cerebellum; Med, Medulla. Created with BioRender.com.

clinical diagnosis, as the questions can be biased toward specific diagnoses, as with the NeP questionnaire. There is little evidence that one survey is the most accurate or effective, and it can be difficult to compare responses across questionnaires.

Clinician scoring systems may be useful, particularly in the context of acute pain. The two most common are the Colorado acute pain score and the Modified Glasgow scale (25–27). Behavioral scales have been developed for cats as well (28). Facial grimace has been validated as a measurement of pain in cats (29, 30). Limitations of these assessments include their reliance on user experience, lack of consideration for patient anxiety, and lack of validation for chronic pain (27, 31).

Studies have validated quantitative measurements of pain, primarily for clinical research. Quantitative sensory testing (QST) has been utilized to test allodynia and hyperalgesia in the context of musculoskeletal and neuropathic pain (32–34). These methods include Von Frey anesthesiometry and cold latency to measure a patient's responses (35–37). These QST techniques can be challenging to administer reliably and therefore may need to be validated by each user (38). Additionally, QST outcome measures are not specific for pain type such as musculoskeletal vs. neuropathic pain and therefore cannot be used in isolation as a measure of neuropathic pain (9, 32).

Actigraphy or accelerometry collars can be used to analyze canine behavior, including to measure aspects of pain (39, 40). In some cases, a simple step count may be useful (40). However, some authors have found there is not a strong correlation between the number of steps and musculoskeletal pain. This may be due to the relationship between an owner's activity and a pet's behavior.

Imaging and electrophysiologic techniques have also been used for pain detection in dogs and cats. For example, in dogs with SM, the location, size, and distribution of the syrinx, as visualized by MRI, can predict the presence of pain (41). In degenerative lumbosacral stenosis, electrodiagnostics, such as F-waves or cord dorsum potentials, may be useful for detecting dogs with painful radiculopathies and confirming a neuropathic pain diagnosis (42).

6 Current research gaps and opportunities

Utilizing technology such as video cameras, high-speed internet, and collar accelerometer trackers, we may be able to incorporate information and observations from the animal's daily environment into clinical practice. This is an active area of study in musculoskeletal research, but there are limited

studies in neuropathic pain thus far (40, 43, 44). Understanding similarities and differences between behaviors at home and clinical observations utilizing these technologies would be a meaningful first step toward understanding their utility for diagnosing neuropathic pain in companion animals.

The relationship between sleep and pain is of interest in humans (see below) as poor sleep and progression of chronic pain are linked (45, 46). Several recent studies have shown effective methods to measure sleep in dogs. This includes developing questionnaires for owners and simplified methods to place electrodes for polysomnography (47, 48).

Although QST is fairly well described, this group of methods currently has limitations due to the high interobserver variabliity, and the variety of techniques used to perform the QST (32, 35, 36, 38, 49). Improving methods of QST and making them more accessible would be beneficial.

Imaging techniques have already been applied to diagnose pain in dogs with SM, where specific imaging features are correlated with chronic pain (50). However, it is difficult to correlate the degree of pain and relevant imaging findings in dogs with degenerative lumbosacral stenosis or intervertebral disc disease, though these both frequently cause neuropathic pain. Perhaps imaging the brain of animals with neuropathic pain may be useful, even if the brain is not considered the primary source of the pain. In humans, chronic pain is correlated with changes in specifics areas of the pain matrix regions of the brain.

7 Cross-species comparisons and opportunities

Over 20% of the US human population experiences chronic pain (51). Chronic pain is defined as pain that occurs either most days or every day and lasting 3 months or longer. The diagnosis of chronic pain in human medicine also relies on a through history including duration of pain, historical injuries, or previous painful episodes. A visual analog scale or numerical score is solicited to determine the perceived severity of pain, although the perception of pain is a subjective measure that differs between individuals (52). The clinician will also question the human patient as to the characteristics of pain, such as tingling, sharp pain, numbness, or burning.

The medical community has recognized the importance of biopsychosocial factors of pain in humans (53). This indicates that the experience and impact of pain in humans is not simply related to tissue trauma. Screening for other psychosocial factors, such as coping behaviors, drug addiction, social support, sleep quality/disorders, and environment, is performed (54). The presence of these risk factors may affect the risk for developing chronic pain as well as the response to treatment. One specific example of a biopsychosocial factor from humans that could be studied in dogs is sleep. Sleep abnormalities are correlated with chronic pain, and insomnia is common (46). Interestingly, sleep impairment is also predictive of worsening chronic pain over time (45). A better understanding of the relationship between sleep and pain in animal could aid in monitoring chronic pain.

Rodents are the most commonly used model of pain for translational research. They are utilized for research into the pathophysiology and treatment of pain. The advantages of rodents are significant and include accessibility to genetic manipulation and repeatable pain models. Similar to humans, there are social and behavioral factors in rodents that affect pain behaviors, such as social housing, stress, or being housed with other animals that are in pain (55). These factors may increase (hyperalgesia) or in some cases decrease a pain response, in the case of stress-induced analgesia (55). The method of testing may also affect pain responses. Factors such as habituation time and handler experience are also important in pain testing. Testing for pain in clinical practice is subject to variables that are often outside of our control, such as transportation or being handled by strangers. However, considering these social factors in pets is likely important when trying to grade and localize neuropathic pain.

The measurement of pain in rodents is accomplished in several ways, such as Von Frey Filament testing and temperature testing. Recent methods of video analysis in rodents have altered the way that we think about pain measurement, as this has helped us expand from reflexive or evoked pain behaviors (of which QST is an example) to also studying spontaneous behaviors. By quantitatively evaluating spontaneous behaviors, we may better understand pain behaviors and response to analgesics (57, 60). Specific behavioral responses such as the trajectory of the paw during withdrawal assays in mice are strain specific, and this type of behavioral analysis may be interesting to study pain responses in different breeds of dogs (56).

8 Discussion

There are opportunities for improving the diagnosis and treatment of chronic neuropathic pain in dogs and cats. When we study the well-defined categories of neuropathic pain in humans, there are some categories that are easily recognized by veterinarians, such as painful radiculopathies and chronic central neuropathic pain associated with spinal cord injury. However, other categories of neuropathic pain reported in humans are not well understood in veterinary species. It is important to recognize that other categories of neuropathic pain, for example disorders that causes painful polyneuropathies, may be present in our veterinary patients. Currently, we may miss some forms of neuropathic pain. For example, if animals are experiencing paresthesias or dysesthesias that cause tingling or numbness, we as clinicians may fail to recognize those animals as experiencing neuropathic pain with any of the currently available metrics.

Priorities for improved pain measurement include improving the detection of pain in the context of anxiety, the stress of veterinary visits, other behavioral changes, or dysphoria. It is interesting to consider that anxiety or stress may be associated with chronic pain in humans while it may mask the diagnosis of chronic pain in companion animals.

We likely need to develop multiple new methods to measure chronic pain and neuropathic pain, as there is a diversity of causes and locations. Ideally, these methods will have high sensitivity, with the ability to discriminate painful and non-painful animals in a clinically useful manner, and each must be carefully validated. Some opportunities to improve chronic pain detection include owner questionnaires, behavioral analysis through video or actigraphy, advanced imaging, and electrodiagnostic techniques. Ultimately, we need methods that are easy to implement into clinical practice,

so that they can be applied to our dog and cat patients and identify patients that would benefit from treatment.

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Conflict of interest

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The authors declare that no Generative AI was used in the creation of this manuscript.

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Diffusion tensor imaging for detecting biomarkers of idiopathic epilepsy in dogs

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Idiopathic epilepsy (IE) is the most common neurological disease in dogs. Approximately 1/3 of dogs with IE are resistant to anti-seizure medications (ASMs). Because the diagnosis of IE is largely based on the exclusion of other diseases, it would be beneficial to indicate an IE biomarker to better understand, diagnose, and treat this disease. Diffusion tensor imaging (DTI), a magnetic resonance imaging (MRI) sequence, is used in human medicine to detect microstructural biomarkers of epilepsy. Based on the translational model between people and dogs, the use of DTI should be investigated in a veterinary context to determine if it is a viable resource for detecting microstructural white matter abnormalities in the brains of dogs with IE. As well, to determine if there are differences in white matter microstructure between dogs who are responsive to ASMs and dogs who are resistant to ASMs. Using DTI to better understand neurostructural abnormalities associated with IE and ASM resistance might help refine diagnostic approaches and treatment processes in veterinary medicine.

KEYWORDS

idiopathic epilepsy (IE), dogs, neuroimaging, biomarker, microstructure, diffusion tensor imaging (DTI), drug resistant epilepsy (DRE), fractional anisotropy (FA)

1 Introduction

Epilepsy is one of the most common neurological diseases in dogs (1). It reduces quality of life and shortens a dog's lifespan (1–5). In veterinary medicine, idiopathic epilepsy (IE) is diagnosed indirectly on criteria that excludes evidence of alternative diseases (6). Therefore, finding biomarkers that could further narrow down the diagnosis of IE would be clinically useful. One option, diffusion tensor imaging (DTI), is used in human medicine to help researchers and doctors better understand brain connectivity and diseases that affect white matter such as epilepsy in people (7–21). Epilepsy arises naturally in both people and dogs; strong arguments exist as to the similarity of the disease between the two species (22–26). Thus, DTI may offer an opportunity to detect microscopic neurostructural abnormalities in dogs that support the diagnosis of IE and improve treatment planning. Furthermore, DTI-detected abnormalities may partially explain why some dogs are responsive to anti-seizure medications (ASMs) while others are resistant as differences have been reported in a couple of clinics trials on people (14, 19). The use of DTI for investigating white matter abnormalities in dogs with IE is in the early stages of exploration (27). In this narrative review, background information will be presented on epilepsy and DTI, and approaches for DTI use, as mostly seen in human medicine, will be explored in the context of veterinary medicine.

1.1 Epilepsy

A seizure is a transient and abnormal increase in synchronization between neurons. Seizures are epileptic when two or more episodes occur at least 24-h apart (28). Seizure types can be described as generalized, focal, or unknown based on their cortical origin. The term 'generalized seizure' refers to synchronized neuronal activity originating in networks that engage both hemispheres. A 'focal seizure' refers to seizure activity originating in one hemisphere. Focal seizures can evolve to bilateral tonic–clonic seizures when activity progresses to generalized activity within a seizure episode. Lastly, unknown seizure type occurs when the hemispheric location of onset of seizure activity is unknown (28, 29). Epilepsy is an enduring predisposition to the occurrence of epileptic seizures. In people, it is classified by seizure type, with implications as to comorbidities, therapeutic recommendations, and outcomes (30, 31).

The focus of the present review is on canine IE; in other words, epilepsy with a known or suspected genetic influence or an unknown cause (6, 28). Similarly, in human medicine, when genetics are known to play a role, IE is referred to as genetic epilepsy. Human medicine has a wider range of defined epilepsy types and syndromes within IE (30, 31). Veterinary medicine is working towards the development of syndrome specificity within IE (32, 33).

There are three tiers of confidence for diagnosing IE in dogs. Tier I is used when two or more epileptic seizures occur at least 24 h apart, signs of epilepsy appear between approximately 6 months and 6 years of age, neurological examination during interictal periods is normal, and baseline blood analysis and urinalysis are unremarkable. Tier II confidence is used when Tier I criteria is fulfilled and no underlying causes are detected using additional blood tests, urinalysis, bile acids tests, cerebrospinal fluid analysis, and magnetic resonance imaging (MRI) of the brain (6). Although standard MRI sequences for the epileptic canine brain are expected to be unremarkable, a few reports exist of hippocampal atrophy or other qualitative or quantitative abnormalities on routine MRI sequences (34, 35). However, it is important to differentiate between post-ictal and interictal abnormalities (36). Cases of post-ictal changes, mainly localized to the piriform lobe, temporal lobe, cingulate gyrus, and hippocampus, have shown a marked reduction to full resolution on follow-up MRIs (36–38). Interictal parenchymal abnormalities are more common among older dogs with IE in regions such as the frontal lobe, piriform lobe, and occipital lobe (39). Tier III diagnostic criteria include all criteria from Tier II plus evidence of seizure activity using electroencephalography (EEG). While a standard protocol for electrode placement exists in human medicine, veterinary medicine is still working towards verifying electrode placement for adequate coverage of the superficial cortical layer in dogs (6, 40–44). Even so, EEG data of an ictal event or interictal epileptogenic patterns still offers the highest level of confidence in the diagnosis of IE (6). This has its challenges as there is a lower likelihood of capturing an ictal or interictal electrographic event in dogs with less frequent seizures (41). Having a microstructural marker that is not reliant on real-time events during neuroimaging would provide another method for obtaining diagnostic confirmation during interictal periods.

1.1.1 Drug resistant epilepsy

While most individuals with IE are successfully treated using ASMs, approximately 25 to 35% of people and dogs have drug resistant epilepsy (DRE) (45–50). The International League Against Epilepsy defines DRE as the failure to reach seizure freedom using two or more ASMs (51). In clinical trials for veterinary medicine, DRE is often referred to as the inability to reduce seizure frequency by at least 50% using two or more ASMs (45–48, 52, 53). The more ASMs being taken, the higher the probability of experiencing unpleasant adverse effects (54). While people might verbally report adverse effects, veterinarians must rely on caregiver observations, behavioral signs, and physiological signs to detect side effects in dogs (6, 53). Therefore, detecting adverse effects is more difficult in dogs than in people. One example of a polytherapy adverse effect that may go unnoticed in dogs versus people is cognitive dysfunction (8). While alternatives to polytherapy are being explored, the overall task of treating and controlling DRE remains a big challenge for both species (3, 53–55).

1.2 Diffusion tensor imaging (DTI)

Diffusion tensor imaging is an MRI sequence based on an algorithmic model. It incorporates data from diffusion weighted images (DWI) taken in multiple planes to form a three-dimensional image. Diffusivity of water molecules is used to highlight structural connectivity patterns of large white matter tracts (Figure 1) (11). Diffusion patterns in the brain are dependent on the density, permeability, and the direction of axons, large molecules, and microstructures.

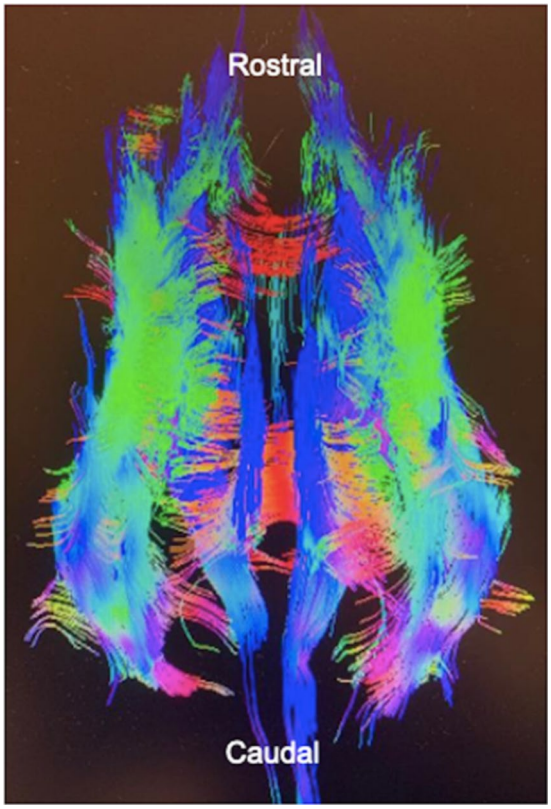

FIGURE 1
A diffusion tensor image of a dog's brain. Tractography is used to predict fiber tract direction which is represented by color. Created with General Electric AW VolumeShare 7.

Diffusion can be referred to as isotropic or anisotropic (Figure 2). Isotropic diffusion is the movement of molecules in an outwards and spherical direction in the absence of structural barriers. The mean magnitude of isotropic diffusion is measured using an apparent diffusion coefficient (ADC). The mean diffusivity (MD) is calculated using ADC values in three orthogonal or more directions. Higher values for these indices are related to increased extracellular space, less structural organization, and fewer axons (11, 21). Anisotropic diffusion refers to the tracking of water molecules within and along densely packed axonal tracts, i.e., the parallel movement of water molecules. Diffusion tensor imaging uses the magnitudes of multiple ADC values in three or more planes to measure the proportion of fractional anisotropy (FA) within a region of interest (ROI). A higher FA value indicates structural organization and a dense region of parallel axons. Dense regions of parallel axons make up large white matter tracts (11, 21, 56).

Diffusion indices can be measured in segments of tracts, tracts, and whole brain white matter. Connectivity indices are used to structurally or functionally analyze networks. Quantitative information on network structure and function can be extracted from DTI using mathematical approaches such as graph theoretical analysis and independent component analysis (14, 19, 57, 58). Indices calculated from graph theoretical analysis highlight the strength, length, and type of connections being made between regions of the brain (19, 58). Independent component analysis allows for the breakdown and buildup of spatiotemporal activity of each voxel (14, 57). Both are useful for understanding brain connectivity and differentiating between normal and abnormal structure or function (14, 19, 57, 58).

Regardless of whether segments, tracts, whole brain white matter, or networks are used as ROIs, each method has benefits and pitfalls (59–61). Segments of white matter tracts allow the selection of regions with lower levels of crossover but are limited by a smaller number of voxels. Conversely, thin slices on image acquisition with zero spacing between slices could be used to increase the number of voxels being selected. Crossover refers to fiber tracts going in different directions within the same voxel and is inevitable when analyzing tracts and whole brain white matter (60, 61). Qualitatively, the overlap from crossing fibers makes it hard to visually differentiate tracts using FA maps, color orientation maps, and tractography. As FA is a vector, and the magnitude of its value is dependent on direction, when tracts cross because they are going in different directions, FA values are cancelled out (59–61).

Tracts and whole brain white matter can also be analyzed using tract based spatial statistics (TBSS); a reliable method for calculating accurate FA values. This method requires distorting, or transforming, each participant's brain images to fit a standard anatomical reference; because of the varying skull morphology in dogs, this task can be challenging and labor intensive but is possible for mesocephalic dogs using a brain atlas space (62). Network measures account for the broad impact of epilepsy but may miss subtle nuances. Furthermore, functional connectivity measures are limited to resting state networks, such as the default mode network, in dogs as they almost always require anesthesia for MRI scans. Resting state networks refer to baseline connections between different functional regions of the brain. These networks highlight brain activity that does not require awareness to elicit measurable changes (63–66). However, it is possible that resting state networks are being functionally altered by anesthesia (64–66).

2 Diffusion tensor imaging in people with epilepsy

People with various types of epilepsy show a trend of decreased anisotropic diffusivity and/or an increase in isotropic diffusivity. Examining patients with generalized genetic epilepsy (GGE), decreased FA and increased MD was found in the corpus callosum, corticospinal tract, superior and inferior longitudinal fasciculus, and supplementary motor areas (12). Similarly, low FA and high perpendicular diffusivity (an additional measurement of isotropic diffusivity) were seen in the posterior corpus callosum, external capsule, internal capsule, and anterior corpus callosum of patients with temporal lobe epilepsy (TLE) (7). Temporal lobe epilepsy is a common form of focal epilepsy in people where activity originates in the temporal lobe (67). Results from these studies imply that the

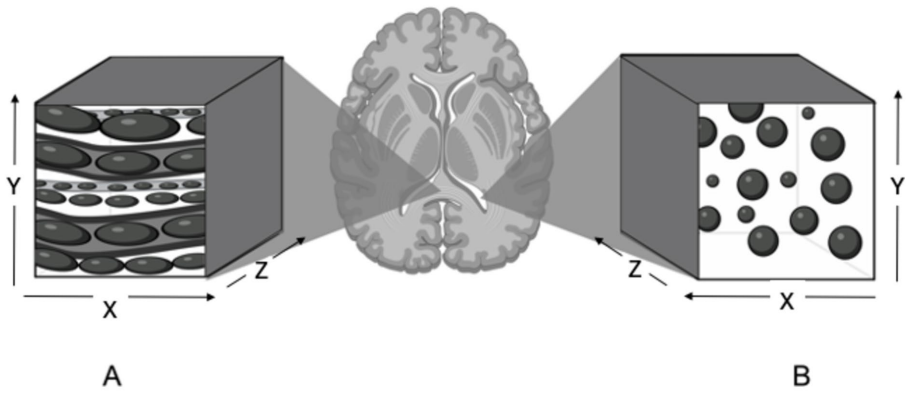

FIGURE 2
Illustration of anisotropic and isotropic diffusion in selected regions of interest in the brain. **(A)** Anisotropic diffusion observed in the corpus callosum. Water molecules are restricted by dense axons and diffuse in a parallel and elongated fashion. **(B)** Isotropic diffusion observed in the lateral ventricle. Water molecules move in an outwards and spherical manner in the absence of structural barriers. Created with BioRender.com.

white matter regions in those with GGE and TLE have more extracellular space and less dense axonal tracts. These studies, among others, highlight the widespread impact that GGE and TLE can have on the brain. As well, they feed into the growing consensus that epilepsy influences or involves structures outside of epileptogenic zones, the predicted origin of seizure activity (7, 12, 17, 68–71).

While white matter structures outside of epileptogenic zones show reduced FA in people with epilepsy, the closer to the epileptogenic zone, the larger the FA reduction. This means there tends to be lower FA in the hemisphere containing the epileptogenic zone (7, 15, 18). Similarly, an ipsilateral reduction in FA is seen in the hippocampal-thalamic pathway of individuals with TLE who experience generalized seizures when compared to controls (10). Another study found an increase in the ADC of the hippocampus located in the hemisphere that initiates seizure activity in those with IE (72). This suggests asymmetry between hemispheres is detectable and should be considered a potential variable in DTI studies of IE. It also suggests that the more brain regions are exposed to seizure activity the more effects are seen, meaning seizure frequency should be considered also as a variable that influences brain microstructure.

People with TLE and hippocampal sclerosis (TLE-HE), TLE, and GGE, show a reduction in FA in multiple white matter regions with the lowest FA values in those with TLE-HE (71). Hippocampal sclerosis refers to the loss of cells within the hippocampus (70). Age of onset and duration with epilepsy is negatively correlated with FA in people with TLE but to a greater degree in those with TLE-HE (7, 71). This implies that the age of onset may influence the extent to which epilepsy changes the structural integrity of regions of white matter that are still developing. The progressive decrease in FA seen in those with TLE, in combination with the greater extent of damage and higher correlation between duration and FA reduction in patients with TLE-HE, could imply quantitative changes are detectable prior to visual detection of structural abnormalities in those with IE.

Functional and structural connectivity abnormalities of neuro-networks are detected in people with IE (14, 19, 20). Functionally, a decrease in connectivity in the default mode network is reported in people with ASM-resistant generalized IE when compared to healthy controls (14). Independent component analysis identified functional connectivity changes in people with juvenile myoclonic epilepsy (JME). There was enhanced connectivity between the prefrontal cortex and the motor cortex, as well as between the supplemental motor areas and lateral and caudal regions of the brain. Supplemental motor areas also showed a decrease in connectivity with rostral regions of the brain (20). Juvenile myoclonic epilepsy is a prominent IE syndrome in humans that shares parallels with JME in Rhodesian Ridgeback dogs (20, 32, 33).

In terms of structural connectivity, there are differences in whole brain networks between people who are good responders versus poor responders to ASMs. Newly diagnosed people with focal epilepsy, naïve to ASMs, had DTI scans prior to determining if they were good-responders to ASMs (IE+) or poor-responders to ASMs (IE-). To meet the criteria for IE+, participants needed to become seizure free for 6 months or more. The mean assortative coefficient, calculated using graph theoretical analysis, was positive for IE+ and negative for IE-. In other words, good responders had more connections between similar brain regions and poor responders had more connections between dissimilar brain regions (19). This provides evidence of microstructural differences in white matter between people who are responsive versus resistant to ASMs.

This body of literature exemplifies a wide range of abnormalities that can be detected using DTI in various presentations of epilepsy (7–21, 68–73). Given the similarities between epilepsy in people and dogs, research from human medicine provides a methodological starting point for what should be explored in veterinary medicine. Conversely, the translational value of canine studies means that they can serve as a valuable source of insights for human medicine (22–26).

3 Translation of DTI literature in people to dogs

People and dogs have similar neuroanatomy and, further, share many aspects of epilepsy such that each could be considered a model for the other (22–26). For example, because the proportion of those with DRE is similar in people and dogs, it is possible that the mechanisms responsible for drug resistance are similar between species (3, 9, 19, 53, 63). More research would be needed to factually support this statement. As DTI is a relatively new technique used to analyze connectivity within the brain, and most published research on DTI and epilepsy has been studied in people, trends from human medicine provide a framework for designing DTI studies for dogs with IE (7, 11, 14, 17, 19, 74).

Conversely, there may also be limits to the transferability of data between human medicine and veterinary medicine. For example, there is a significant difference between the connectivity of the anterior cingulate cortex and posterior cingulate cortex when comparing neurotypical people and dogs. More specifically, the dogs had lower anisotropic diffusion between their anterior and posterior cingulate cortex (57). Epilepsy in people has been correlated with lower anisotropic diffusion in multiple white matter structures; thus, using human medical literature to interpret canine data may result in inaccurate conclusions (14, 57, 63). Therefore, overall trends from human medicine should be considered but actual FA values of ROIs are not reliable measures to compare between the species.

3.1 Selecting regions of interest for dogs with idiopathic epilepsy (IE)

Epilepsy affects the microstructure and function of white matter tracts and influences the connectivity patterns within and between neuronal networks. Based on this, it is reasonable to use either segments of tracts, tracts, whole brain white matter, or networks as ROIs (7–21, 68–72).

Segments of white matter tracts investigated in people with IE include the corpus callosum, cingulum, external capsule, internal capsule, mammillothalamic tract and hippocampus (7, 12, 17, 69–73, 75). Depending on breed, MRI quality, and software constraints, the external capsule and mammillothalamic tract may be too small for voxel selection in dogs. However, the corpus callosum, cingulum, internal capsule and hippocampus are prominent structures that could be used as ROIs for dogs (27, 56, 76).

Whole tracts studied in people with IE that could be used as ROIs in dogs include the corticospinal tracts, superior longitudinal fasciculi, interior longitudinal fasciculi, and hippocampal-thalamic pathway

(10, 12, 17, 74). In dogs, the corticospinal tract is hard to differentiate from the corticobulbar and corticopontine tracts meaning these may need to be analysed together (56).

The default mode network and overall brain networks could be used as ROIs when studying functional and structural connectivity (14, 17, 19). While other networks may be involved in epilepsy, such as the thalamocortical network or basal ganglia, these regions would require functional MRI techniques and unanesthetized dogs, making for a more complex experimental protocol and timeline (57, 63, 77).

4 Diffusion tensor imaging in dogs

As DTI is a relatively new technique, only a few studies have demonstrated the feasibility of its use in a veterinary context (27, 56, 63, 78–81). Extensive mapping of separate white matter tracts in healthy dogs using DTI has been verified with cadaveric dissections and anatomy textbooks, creating atlases. Such atlases could be used to compare tracts between healthy dogs and dogs with IE (56). Other atlases focused on parcellation of subcortical and cortical grey matter regions. While individual tracts and structures of white matter were not parcellated, segmentation of whole white matter was defined (62, 79, 82–86). Grey matter abnormalities are less of a focus in DTI but a significant increase in ADC in the piriform lobes of dogs with IE has been reported (87). Meaning, parcellation of these grey matter regions could be useful for DTI research. Furthermore, identifying both grey and white matter abnormalities comes into play in regions like the hippocampus, a portion of the piriform lobe with a mixed composition of grey and white matter. In terms of whole brain white matter, at least one of these atlases are being used to standardize participants' brains for TBSS (62).

An increase in isotropic diffusivity and decrease in anisotropic diffusivity in the hippocampus of people with TLE without HE provides evidence that quantitative imaging may precede visually recognizable structural abnormalities (71, 72). Similarly, in a study on dogs with IE, there was an increase in hippocampal atrophy, from 12 to 36%, when calculated visually versus using the hippocampal asymmetry ratio (35). Hippocampal atrophy is sometimes present on MRI scans in relation to IE which is a discrepancy with IVETF tier II criteria stating that there are no structural brain abnormalities in dogs with IE (6, 34). The hippocampal asymmetry ratio is dependent on volumetric measures. Therefore, other quantitative measures, such as diffusion indices, may also be sensitive to microstructural abnormalities in the brains of dogs with IE.

Asymmetry between hemispheres may be a feature to explore in dogs with IE. Notably, a certain amount of FA asymmetry is to be expected in some regions based on what is reported in people. For example, the left corticospinal tract tends to have higher FA than the right corticospinal tract in healthy people (74). The corticospinal tract has been reported as an ROI for FA reduction in people with IE (12). Therefore, a thorough understanding of asymmetry in healthy individuals would need to be investigated prior to making any conclusions related to IE in dogs.

The other route for studying microstructural and functional abnormalities in dogs with IE is to look at DTI formulated probabilistic representations of neuro-networks (14, 19, 57, 58). Graph theory analysis could be applied to DTIs in dogs with IE, using methodological frameworks published in human medicine, to enable the investigation of structural abnormalities of connectivity (19). Functionally, the anterior region of the default mode network in dogs with IE shows an increase in connectivity (57). In this example, results differed from a similar study in people with general IE (14). While these results are contradictory, it was theorized that there may be a compensatory increase in connectivity prior to degradation that occurs over the course of the disease (57). The same concept was discussed in a study that found an increase in FA in the frontal lobe of newly diagnosed and treatment naïve children with generalized IE (16).

4.1 Diffusion tensor imaging in dogs with IE

Only one article has looked at DTI in dogs with IE. Notably, the authors selected a couple ROIs that have been found to have reduced FA in people with epilepsy, namely, the corpus callosum and cingulate white matter. They reported a significant decrease in FA in the cingulate white matter of dogs with IE. Tract based spatial statistics of this cingulate white matter did not show the same significant decrease in FA which may be because TBSS does not highlight subtle microstructural differences (27). These findings could be expanded by using a larger population and additional ROIs.

5 Additional variables to consider for DTI research in dogs with IE

Breed and age affect brain microstructure in canines and sex and age affect brain microstructure in humans (7, 17, 81, 88–92). Morphological variability exists between dog breeds, where specific behaviors correlate with structural differences in the brain (89). Moreover, human studies have shown differences in brain diffusion and connectivity between sexes. For example, one study reports that males have higher FA in multiple white matter regions when compared to females (91). Another large study found males have more intrahemispheric connections while females have more interhemispheric connections (90). In terms of age, neuronal degradation in people, which is reflected by a decrease in FA, generally starts in the anterior region of the brain and continues posteriorly (92). Therefore, age at scan may negatively correlate with FA in certain regions of the brain (7, 81, 92). In relation to dogs, Barry et al. (81) studied the influence of age on white matter FA in a sample of 29 healthy mesaticephalic dogs. The dogs were divided into two age categories: the young group included dogs under the age of 7, and the old group included dogs aged 7 or older. They found a significant decrease in FA in multiple white matter regions, including the corpus callosum, in the old group when compared to the young group (81). Note, a decrease in FA in relation to age is not noticeable before the age of 30 in people (88); meaning, a pattern may not be evident in a sample of mainly young dogs. Overall, breed, sex, and age should be considered when determining the relationship between white matter microstructure and IE in dogs.

Volume is another factor to consider when studying IE in dogs. While volume is not the focus of this review, volumetric data is simultaneously provided when selecting ROIs from DTIs for diffusion analyzes (93). Nuyts et al. (17) conducted a meta-analysis on structural abnormalities associated with generalized IE in people. They reported

a statistically significant reduction in volume in the supplemental motor area, insula, thalamus, putamen, caudate, hippocampus, anterior cingulate cortex, and left pallidum. Also, the medial frontal gyrus was larger in the right hemisphere of those with generalized IE compared to controls (17). Furthermore, Milne (94) reported that dogs with IE had a widespread reduction in cerebral cortical volume when compared to healthy controls (94). While volume of structures can vary between individuals, overall volumetric patterns and ratios are worth investigating as all the information needed would already be obtained (17, 89).

6 Discussion

The question of whether DTI is a viable resource for detecting microstructural white matter abnormalities in the brains of dogs with IE needs further investigation. Comparisons using DTI indices have yet to be conducted between dogs with IE+ and IE−. A prospective case–control cohort study design would be optimal for determining whether there are microstructural differences between healthy dogs, IE+, and IE−. Healthy dogs and dogs with newly diagnosed IE, naïve to ASM treatment, could receive an initial scan and a follow up scan 12 months later. During these 12 months, dogs with IE would be treated with ASMs and categorized as ASM responsive or resistant following IVETF definitions. Diffusion indices and structural connectivity measures could be blindly analyzed. Comparisons could be made (1) between neurotypical dogs and dogs with IE to control for confounds; (2) between the initial scans of dogs that are resistant versus responsive to ASMs to determine the presence of biomarkers; (3) as well as between and within arms over time to account for additional confounds and compare progressional changes in diffusion and connectivity of the brain.

The first comparison controls confounds such as breed, sex, and age. The second comparison would be useful for determining whether microstructures detected by DTI could be used as biomarkers to help predict ASM resistance; in turn, adding more specification to diagnoses and allowing for more informed treatment planning. Structural connectivity has been compared between people who are good versus poor responders to ASMs (12). Assortative connectivity was associated with good responders and disassortative connectivity was associated with poor responders. With the similarities between human and canine epilepsy, these results may translate to dogs. The range of structural networks that have been explored using DTI in humans is vast, whereas the most practical functional networks to investigate in dogs with IE are resting state networks (14, 19, 57, 63–66). In addition to network measures, anisotropic diffusivity in segmentations of tracts, tracts, or whole brain white matter could be investigated. Segments of tracts previously implicated in human IE that are translatable to dogs include the corpus callosum, cingulate gyrus, internal capsule, and hippocampus. Furthermore, tracts include the corticospinal, corticobulbar, and corticopontine tracts, the superior and inferior fasciculi, and hippocampal-thalamic pathway (7, 10, 12, 17, 69–71, 73–75). If diffusion indices are investigated, Figley et al. (95) emphasizes the importance of using multiple measures to validate FA findings. This is because the ratio of neurons going in the principal direction is being calculated, not overall density of fibers within a voxel. For instance, FA could be higher in an area with fewer parallel fibers than in an area with densely packed fibers going in multiple directions. Also, damaged white matter may not result in a change in FA if fibers in all directions are equally damaged; this is because the ratio between the fibers going in different directions would remain the same (95). Furthermore, histological findings suggest that ADC, an isotropic diffusion index, is a better measurement of myelination than FA, as FA measures myelination among other microstructural constraints of water diffusion. Different diffusion indices offer some overlap and some variation in information (95–97). Therefore, more detailed information can be obtained using multiple diffusion indices when studying IE.

Thirdly, asymmetry comparisons over time between hemispheres should be made as differences have been exemplified in people and dogs with IE (7, 15, 18, 35, 71, 73). More importantly, it would provide information about the progression of changes in ASM responsiveness versus resistance in patients. As well, act as a within control measure to gain pilot data for future investigations on additional factors that are potentially influencing microstructural change such as number of ASMs being taken, ASM type, seizure frequency, and seizure type.

7 Conclusion

Research is building on evidence of microstructural abnormalities in dogs with IE from DWI, volumetric studies, and one DTI study (27, 35, 87, 94). Functional abnormalities in the default mode network of dogs with IE have been identified (57). Further investigations will be needed into anisotropic diffusivity and structural connectivity measure in dogs with IE. While DTI is a useful tool to investigate brain abnormalities related to epilepsy in people, findings from human medicine are not always transferable to veterinary medicine (7, 11, 12, 14, 17, 19, 57, 73). On this front, human medicine is ahead of veterinary medicine and provides a valuable framework to guide veterinary research. In turn, veterinary medicine may provide valuable information for human medicine (22–26). There are many types of ROIs and measures that could be used, and each one comes with benefits and limitations. As well, confounds such as breed, age, sex, asymmetry, ASM specifications and seizure or epilepsy type specifications would need to be considered. In tandem with other diagnostic techniques, discoveries using DTI could lead to more specific diagnoses and targeted treatments for dogs with IE (12, 17, 19, 32, 33, 57, 95, 97).

Author contributions

GK: Writing – original draft, Writing – review & editing, Conceptualization, Data curation, Investigation, Methodology. AZ: Writing – review & editing, Methodology, Resources. LG:

Writing – review & editing, Resources. FJ: Conceptualization, Writing – review & editing, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Neuroanatomy of spinal nociception and pain in dogs and cats: a practical review for the veterinary clinician

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Chronic pain is a prevalent condition in companion animals and poses significant welfare challenges. To address these concerns effectively, veterinary clinicians must have a comprehensive understanding of the neuroanatomy of nociception and the intricate processes underlying pain perception. This knowledge is essential for planning and implementing targeted treatment strategies. However, much of the existing information on pain mechanisms is derived from studies on rodents or humans, highlighting the need for further translational research to bridge this gap for veterinary applications. This review aims to provide veterinary clinicians with an in-depth overview of the spinal nociceptive pathways in the dog and cat, tracing the journey from nociceptor activation to cortical processing in the brain. Additionally, the review explores factors influencing nociceptive signaling and pain perception. By enhancing the understanding of these fundamental physiological processes, this work seeks to lay the groundwork for developing effective therapies to manage the complexities of chronic pain in companion animals.

KEYWORDS

chronic pain, maladaptive pain, nociceptive pathways, periaqueductal gray, Rexed laminae, spinocervicothalamic tract, spinothalamic tract, thalamus

Introduction

Effective pain management is a critical aspect of companion animal care. However, translating findings from rodent-based pain research into practical clinical strategies for veterinary medicine remains a significant challenge. The veterinary literature has historically focused more on the assessment and management of acute pain, with chronic pain—apart from osteoarthritis-related conditions—receiving comparatively less attention. This gap leaves veterinary surgeons less equipped to objectively monitor and manage chronic pain conditions in their patients.

Understanding the mechanisms of pain generation and the maladaptive processes that characterize chronic pain is a crucial step in developing effective multimodal treatment approaches. This review aims to provide veterinary clinicians with a comprehensive overview of the neuroanatomy of feline and canine spinal pain pathways. It covers the journey from nociceptor activation through the spinal cord dorsal horn, ascending spinal tracts, and relays to the somatosensory cortex. Additionally, it explores factors that influence pain perception, including spinal and supraspinal modulation, cognitive and emotional influences, and anatomical differences between dogs and cats. By integrating this knowledge, clinicians can improve their ability to manage chronic pain in companion animals effectively.

What is pain?

The International Association for the Study of Pain (IASP) defines pain as "*an unpleasant sensory and emotional experience*," implying that pain is not just a matter of sensation but also a conscious registration and emotional consequence (1). This makes pain a very complex sensation and experience with wide individual variety. In veterinary medicine, there is no veterinary-specific definition of pain; however, the IASP definition of pain states that "inability to communicate does not negate the possibility that a human or a nonhuman animal experiences pain" (1). There is therefore a common understanding that the definition by IASP applies to animals as well as humans.

Pain perception and the nociceptive system are integrated parts of the nervous system that allows an individual to interact with the environment and react accordingly to the received stimuli. In most cases, the purpose of feeling pain is to protect the individual from further harm and facilitate healing (2).

It is important to distinguish between "pain" and "nociception" (coming from the Latin word *nocére*, meaning to injure or harm). *Pain* encompasses a subjective and conscious perception whereas *nociception* refers to the neuronal mechanisms that encode noxious stimuli (1). These two terms are often used interchangeably, as the two phenomena often occur together. However, it is possible to have nociception without pain, as in the case of general anesthesia during surgery (2). Although the body registers and processes noxious stimuli from the surgeon's scalpel, the patient does not consciously perceive the pain. Also, it is possible to have pain without nociception, as in the case of phantom pain described in cats after limb amputation (3).

Nociceptive pain arises from actual or threatened damage to non-neural tissue due to the activation of specialized receptors (nociceptors) in the peripheral nervous system (4). These receptors respond to one or more types of noxious stimuli of mechanical, thermal, or chemical (e.g., inflammatory) origin. Nociceptive pain is the most common type of acute pain but may become chronic (2). Acute pain is often *adaptive* in nature, meaning it ceases to exist once healing is completed, whereas chronic pain is characterized by becoming *maladaptive* because it persists longer than expected (2). Currently, there is no consensus on when pain is considered chronic, but in humans it is commonly accepted that pain that exists for longer than expected for a given injury, or for more than 3 months is considered chronic (2). It has been suggested that this definition should be adapted and shortened for companion animals to reflect their shorter lifespan (5). *Neuropathic* pain is not caused by activation of nociceptors but is due to damage to or disease of the somatosensory nervous system. *Nociplastic* pain is proposed as a term to explain evoked types of pain, in which the patient experiences pain without any apparent reason due to alterations in the somatosensory system (4). Nociplastic pain has been shown to exist in humans and rodent models (6–8). While strong evidence for nociplastic pain in dogs or cats is currently lacking, it is anticipated that this type of pain occurs in these species as well.

From nociception to pain perception

In simple terms, nociceptive pain is initiated by activation of specialized nerve receptors located at the end of axons from nerves originating from the dorsal root ganglion (9, 10). These receptors, known as nociceptors, detect harmful stimuli affecting peripheral tissues and generate electrical signals that are transmitted through the nervous system (Figure 1). Nociceptors are broadly classified based on their responsiveness to various stimuli and may be activated by one or multiple types of stimuli (11). Mechanosensitive nociceptors primarily respond to mechanical stimuli, such as cutting or pressure, while thermal and chemical nociceptors are activated by changes in temperature or chemical signals. Mechanothermal nociceptors are responsive to both mechanical and thermal stimuli, whereas polymodal nociceptors exhibit broad functional diversity, detecting and responding to a combination of mechanical, thermal, and chemical stimuli (11). Nociceptors can be further classified as high- or low-threshold nociceptors based on the intensity of stimuli required to activate them (12). Low-threshold mechanoreceptors (LTMRs) respond to low-intensity mechanical stimuli such as touch, while high-threshold mechanoreceptors (HTMRs) are specialized to detect potentially harmful mechanical stimulation (12). Nociceptive information is transmitted from nociceptors to neurons in the dorsal root ganglion and then directly into the dorsal horn of the closest spinal cord segment or via Lissauer's tract to an adjacent spinal cord segment (13). Approximately 80% of the axons travelling through the Lissauer's tract are unmyelinated fibers (13). In the spinal cord, the neuron from the dorsal root ganglion synapses with either a projection neuron or an interneuron. Interneurons are abundant in the spinal cord and help modulate incoming inputs by either enhancing or reducing them (14). This helps to control the magnitude of the inputs that are projected to the brain. In contrast, projection neurons transmit signals to the brain thereby participating in generating a response to pain (15). Depending on where they terminate in the brain, the response may be either conscious or subconscious (16, 17). The brain can modulate inputs by sending signals to the spinal cord, which can either amplify or diminish noxious stimuli (16, 18). Amplifying nociceptive signals help the animal recognize danger and move away from potential harm. Conversely, reducing these signals can enable the animal to cope during dangerous situations without being overwhelmed by pain. Projection neurons transmit the incoming signal from the dorsal horn to spinal cord pain pathways, connecting this input to various brain structures, such as the brainstem, thalamus, and somatosensory cortex (15, 16). Most nociceptive inputs are transmitted to the thalamus in diencephalon. Serving as a complex yet vital relay center, the thalamus processes sensory signals and redistributes them to the cerebral cortex, where the final conscious perception of pain occurs (15, 16, 19).

The architecture of the dorsal horn

The architectural structure of the dorsal horn was identified by Swedish neurologist Bror Rexed in the 1950s studying spinal cords in cats (20, 21). He described it as being organized into six layers, or laminae (I-VI), each containing different cell types, compositions, and functions crucial for processing and transmitting various types of sensory information (11, 12) (Figure 2). The most dorsal layer, lamina I along with the second layer, the *substantia gelatinosa*, are considered the primary nociceptive regions of the dorsal horn. These layers mainly receive noxious inputs, as well as thermal and touch signals (22). The peripheral afferent fibers can enter the dorsal horn directly

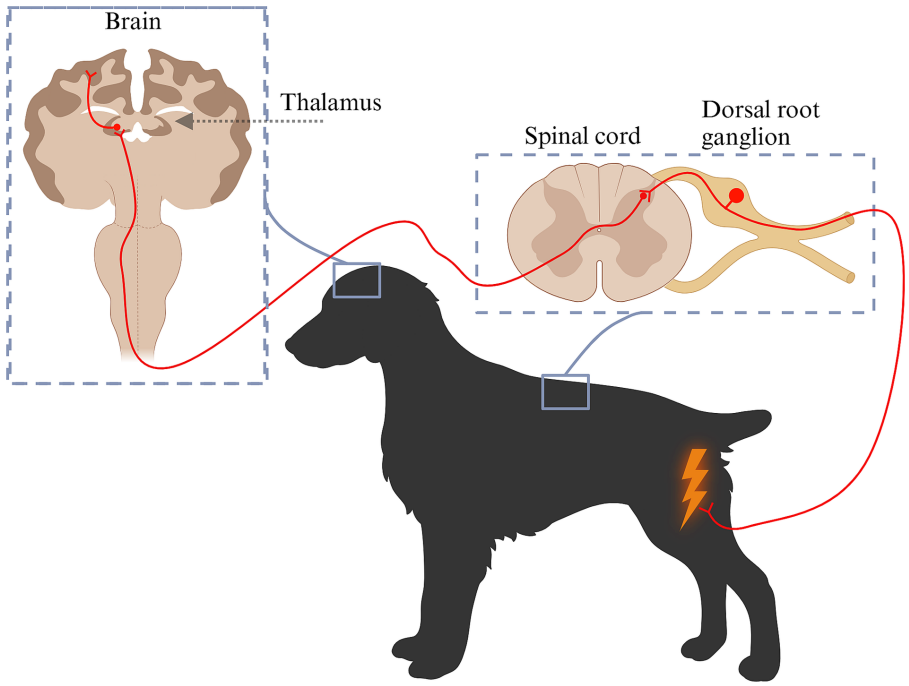

FIGURE 1
Schematic diagram of pain pathways from nociception to pain perception. Pain is initiated by specialized nerve receptors known as nociceptors. Nociceptors become activated by various types of stimuli: mechanical stimuli (cutting, pressure), temperature or chemical changes (e.g., inflammation). The generated signal is transmitted to the neuronal cell body located in the dorsal root ganglion, from where it is relayed to the dorsal horn. Within the dorsal horn, the primary neuron synapses with a projection neuron or an interneuron that works to modulate incoming inputs by either enhancing or reducing them. Projection neurons transmit signals to the brain including the thalamus, from where they are distributed onwards to different brain structures and perceived as pain. Created in BioRender. Pedersen (2025) https://BioRender.com/y80d045.

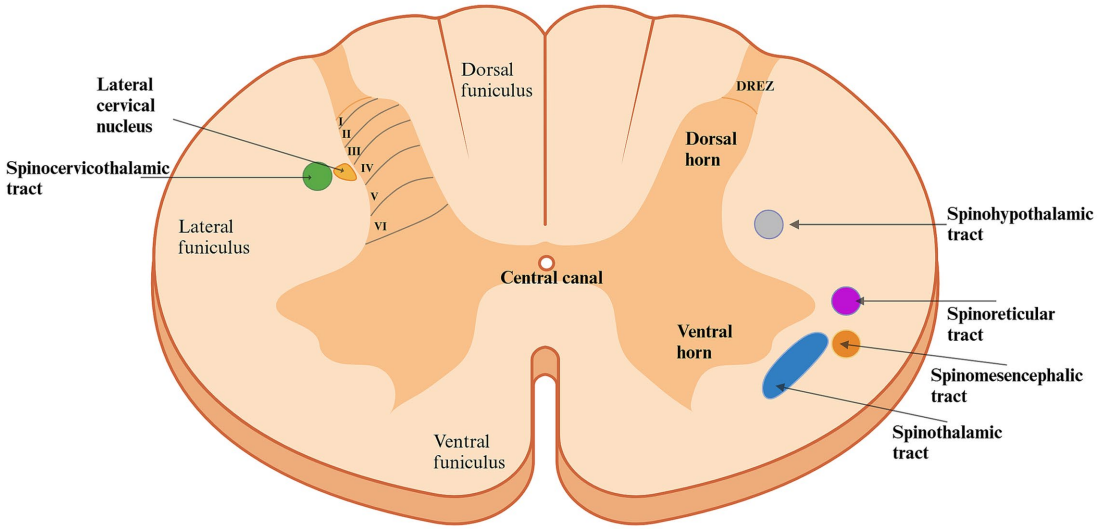

FIGURE 2
Schematic diagram of the architecture of the spinal cord in the first segments of the cervical region. The dorsal horn is organized into six layers (*laminae*), each with different cell types, compositions, and functions essential for processing and projecting various sensory inputs. Laminae I and II predominantly receive nociceptive input. Moving ventrally through the laminae, the complexity increases: Lamina III primarily receives innocuous (non-nociceptive) inputs and contains a high proportion of interneurons. Besides innocuous input, lamina IV also handles nociceptive input and contains a large proportion of projection neurons. Lamina V, located deeper in the dorsal horn, receives input from deeper structures such as the skin, muscles, and joints, whereas lamina VI is predominantly composed of interneurons and proprioceptive neurons forwarding information. Consequently, nociceptive transmission involves several laminae of the dorsal horn, resulting in various nociceptive pathways, each originating from a different area of the spinal cord, carrying specific input to different areas of the brain. The spinocervicothalamic and spinothalamic tracts are described to be the two most significant nociceptive pathways in the dog and cat. Created in BioRender. Pedersen (2025) https://BioRender.com/l36q898. DREZ: Dorsal Root Entry Zone.

through the dorsal horn entry zone or travel up to 2–3 spinal cord segments via the Lissauer's tract before entering (23, 24). These fibers, originating from dorsal root ganglion neurons can synapse directly onto projection neurons in lamina I or onto dendrites of neurons in deeper laminae that extend dorsally into lamina I (25, 26). Projection neurons from lamina I contribute directly to nociceptive pathways, such as the spinothalamic and spinomesencephalic tracts, while intralaminar neurons in lamina II may connect with other intralaminar neurons or with neurons in other laminae (15, 26–29). The nociceptive tracts will be discussed in further detail in the following section. As we move ventrally through the dorsal horn, the complexity of the laminae's composition and function increases. Lamina III primarily receives innocuous (non-nociceptive) inputs and contains a high proportion of interneurons, although it also includes a few spinoreticular and spinocervicothalamic projection neurons (28, 30). Similarly, lamina IV receives input from innocuous stimuli but also contains a significant number of projection neurons associated with the spinocervicothalamic tract. As a result, it also responds to noxious stimuli (31–38). Laminae III and IV are collectively referred to as the *nucleus proprius*. Lamina V, located deeper in the dorsal horn, receives input from deeper structures such as the skin, muscles, and joints. It contains a diverse range of neuronal types, including sensory afferent fibers (predominantly C-fibers), wide dynamic range (WDR) neurons, proprioceptors, interneurons, projection neurons, and neurons receiving inputs from the viscera (28). Most of the neurons are multi-receptive and respond to both innocuous and noxious inputs (15, 28). Some participate in the spinothalamic and spinomesencephalic tract (15). The innermost layer of the dorsal horn, lamina VI, consists primarily of interneurons and proprioceptive neurons, along with some spinoreticular projection neurons (28). This lamina is particularly well-developed in the intumescences, reflecting the heightened proprioceptive input from muscles and joints in these regions, but it is less distinct in other areas of the spinal cord (21).

In addition to the gray matter of the spinal cord, the white matter can also be divided into distinct regions. These regions are called *funiculi* and are named according to their locations (Figure 2) (39). The lateral and ventral funiculi consist mainly of axons from projection neurons, including nociceptive tracts (21, 28, 40).

In conclusion, most layers of the dorsal horn receive nociceptive input and play a role in transmitting these signals to the brain.

Central nociceptive pathways

The path from the detection of noxious stimuli to the perception of pain is not a single, direct route to the brain; rather, it involves multiple nociceptive tracts originating from different regions of the dorsal horn in the spinal cord (Figures 2, 3; Table 1).

The spinocervicothalamic tract

In carnivores, the spinocervicothalamic tract is believed to be the most dominant and biologically relevant nociceptive pathway, followed by the spinothalamic tract (28, 41, 42) (Figure 3). This claim is supported by studies showing that the lateral cervical nucleus, from which the spinocervicothalamic tract projects, is large in carnivores but rudimentary or absent in humans (35, 43). However, there is a lack of robust studies supporting the dominance of the spinocervicothalamic tract in carnivores over other nociceptive pathways, such as the spinothalamic tract. The spinocervicothalamic tract originates from the lateral funiculi of the white matter, just next to laminae III-V across all body regions, projecting input ipsilaterally to the cranial cervical region, where it synapses in the lateral cervical nucleus (20, 38, 44). The lateral cervical nucleus is located in lamina IV between the caudal part of C1 and cranial part of C3 of the spinal cord (38). The neurons of the lateral cervical nucleus receive both innocuous and nociceptive inputs from all parts of the body via the spinocervicothalamic tract. The primary inputs are innocuous tactile signals, which can be triggered by even minor hair movements (31–37). Nociceptive inputs can be triggered by either thermal or mechanical noxious stimuli, such as skin pinching. The receptive fields of these inputs can vary greatly, ranging from very restricted areas (such as a part of a toe) to large or even complete sections of the body (37, 44–46). Conduction velocities vary between 7 and 92 m/s, with glutamate and Substance P serving as key excitatory neurotransmitters, while GABA plays a crucial role in inhibitory neurotransmission (36, 47–53). Signals from the lateral cervical nucleus are transmitted through the dorsolateral funiculus of the spinal cord and ascend through the brainstem, where they decussate to the contralateral medial lemniscus (54–56). These signals primarily terminate in different thalamic nuclei, with the majority ending in the thalamic ventral caudal-lateral nucleus (*nucleus ventralis caudalis*) (54–56). When travelling along the medial lemniscus towards the ventral caudal-lateral nucleus, spinocervicothalamic projecting fibers may branch off and terminate in the midbrain region of the caudal and rostral colliculus (57). In cats, a small percentage of spinocervicothalamic neurons project directly to the periaqueductal gray in the brainstem. The periaqueductal gray is a cell-dense area of gray matter situated centrally around the mesencephalic cerebral aqueduct and plays an important role in descending pain modulation, as well as emotional aspects of pain (58–60). The projection to the periaqueductal gray suggests that the spinocervicothalamic tract may play a role in the descending modulatory pathway or in alerting the animal to potential threats (37, 61, 62).

The spinothalamic tract

The spinothalamic tract is the most important nociceptive tract in humans, and lesions to it may result in neuropathic pain (63). It is considered an important nociceptive pathway in animals as well. Spinothalamic projection neurons originate from all spinal cord regions and lamina I and IV-VII and transmit with high velocities, suggesting rapid communication of potentially harmful stimuli to higher centers in the body (64, 65). In the cervical spinal cord of cats, spinothalamic cells are primarily somatotopically organized within the dorsal horn, with receptive fields like those of the surrounding interneurons (64). The sizes of spinothalamic receptive fields in the cervical spinal cord are smaller and located to the forelimbs compared to the larger spinothalamic lumbar receptive fields located to the hindlimbs (64, 66). Most spinothalamic neurons traverse the contralateral ventrolateral funiculi of the spinal cord, and a minority travel directly through the dorsolateral funiculi (67–70). Both ascend through the brainstem, close to the medial lemniscus, and terminate in multiple thalamic nuclei (64, 67, 71) (Figure 3). Along their pathway, they send out collateral axonal branches that synapse with various brain structures, including the

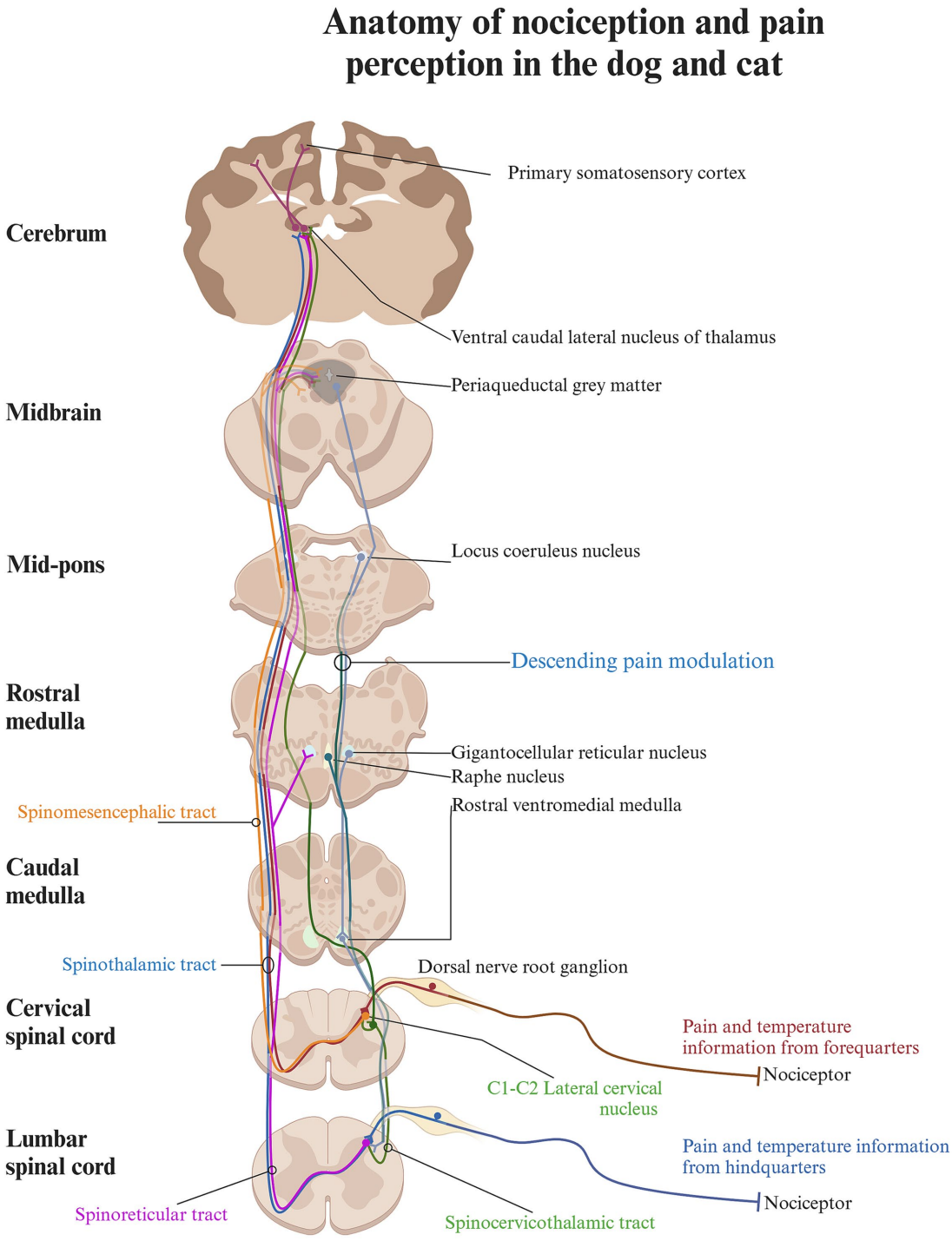

FIGURE 3
Schematic diagram of the anatomy of nociception and pain perception in the cat and dog. The pathway from registering noxious stimuli to perceiving pain involves multiple nociceptive tracts from various regions of the dorsal horn. Each tract transmits different sensory inputs to specific brain regions, contributing to the perception of pain based on a complex set of input. The spinocervicothalamic and spinothalamic tract are contributing to conscious pain perception, as they relay information to higher brain structures via the thalamus, whereas the spinoreticular and spinomesencephalic tract terminates in the brainstem and the reticular formation and plays a role in the descending modulatory system. Created in BioRender. Pedersen (2025) https://BioRender.com/o86g825.

periaqueductal gray and the reticular formation. This suggests a role in descending nociceptive modulation and alertness behavior (70, 71). The spinothalamic cells receive miscellaneous inputs, ranging from pressure, and temperature to somatic and visceral nociception (64, 69, 70, 72–75). Transmission of stimulation from innocuous mechanical input including light touch and innocuous heat has also been described (64, 70, 74, 75). Specific spinothalamic neurons also respond to histamine and contribute to sensations of itch (68).

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TABLE 1 Overview of spinal nociceptive pathways in dogs and cats.

Tract	Origin	Termination	Function	Main neurotransmitters
Spinocervicothalamic tract	Lateral cervical nucleus (located in the white matter outside lamina III-IV in C1 to the cranial part of C3)	Thalamus: Ventral caudal-lateral nucleus	Tactile sensations, nociception	Excitatory: • Glutamate • Substance P Inhibitory: • GABA
Spinothalamic tract	Lamina I + IV-VII	Thalamus: Multiple nuclei including the ventral caudal-lateral nucleus	Nociception, temperature, tactile and mechanical sensations, itch	Excitatory: • Glutamate • Substance P Inhibitory: • GABA • Glycine
Spinoreticular tract	Lamina VI-VIII	The reticular formation: the gigantocellular reticular nucleus	Nociception, tactile and mechanical sensations	Excitatory: • Glutamate Inhibitory: • GABA • Glycine
Spinomesencephalic tract	Lam. I, III-V	The reticular formation: various nuclei and the periaqueductal gray	Nociception, mechanical sensations	Excitatory: • Glutamate Inhibitory: • GABA • Glycine
Spinohypothalamic tract	Spinal cord marginal zone, lateral reticulated area and area surrounding the central canal.	Hypothalamus, thalamus, rostral colliculus, reticular formations	Contributes to affective component of pain including reflex autonomic and endocrine responses to painful stimuli and emotional reponse	Excitatory: • Glutamate Inhibitory: • GABA • Glycine

Several spinal nociceptive pathways, with the spinocervicothalamic and spinothalamic tracts being the most prominent, play key roles in pain perception in dogs and cats. Both tracts transmit nociceptive and tactile signals, while the spinothalamic tract additionally conveys sensory information such as temperature and itch. These pathways are integral to conscious pain perception, as they relay information to higher brain structures via the thalamus. In contrast, the spinomesencephalic and spinoreticular tracts terminate in the brainstem, where they contribute to the modulation of pain through the descending inhibitory system.

Glutamate and Substance P are recognized as key excitatory neurotransmitters of the spinothalamic tract in cats (76, 77). In studies conducted on monkeys, GABA and glycine have been identified as mediators of inhibitory neurotransmission within the spinothalamic tract (78). The role of neurotransmitters is discussed in more detail later in this paper. Studies have shown that spinothalamic cells in cats become sensitized to heat following exposure to intense heat for 30 s (64). Consequently, these cells exhibit a heightened response to lower temperatures afterwards (64).

The spinomesencephalic tract

The spinomesencephalic tract originates from layers I and III–V in the dorsal horn of the cervical and lumbar spinal cord (15, 29). Its neurons respond to both innocuous and noxious mechanical stimulation from sources such as hair, skin, and deeper structures, including muscles and joints (27, 37, 79). It furthermore receives collateral input from the spinocervicothalamic tract (80).

In rats, glutamate has been identified as a primary excitatory neurotransmitter of the spinomesencephalic tract, while glycine and GABA are suggested to play significant inhibitory roles in cats (81–83). Like the spinothalamic neurons, the spinomesencephalic neurons decussate to the contralateral spinal cord to the ventrolateral funiculi. They ascend through the brainstem, terminating in various nuclei within the reticular formation, including the caudal and rostral colliculi and the periaqueductal gray, with a smaller number continuing to the thalamus (11, 29, 58, 62, 79). Electrical stimulation of the termination sites in the periaqueductal gray has been shown to decrease pain in cats, suggesting that the spinomesencephalic tract may also be involved in descending nociceptive modulation or the emotional aspect of pain (29, 58, 79) (Figure 3). The descending nociceptive modulation as well as the emotional aspect of pain are covered in later sections in greater detail. In cats, spinomesencephalic fibers have furthermore been found to terminate in the parabrachial nucleus, a structure that, in rats, has been shown to contain a significant amount of the endogenous opioid enkephalin (29, 84, 85). Enkephalin, a key pain modulator, binds to opioid receptors throughout the body and is widely distributed in the spinal cord and trigeminal nucleus. In cats, it is particularly concentrated in laminae I, II, and V of the dorsal horn (84–86).

The spinoreticular tract

The spinoreticular tract primarily originates from the deep layers of the spinal cord (15, 87, 88). Spinoreticular neurons respond to both innocuous and nociceptive stimuli, which range from light touch and

hair movement to subcutaneous pressure, as well as lifting and pinching of the skin (17). Glutamate has been identified as a main excitatory neurotransmitter of the spinoreticular tract in cats, while glycine and GABA have been shown to play significant inhibitory roles in rats (89, 90). Spinoreticular neurons also project to the contralateral ventrolateral funiculi, travelling close to the axons from the spinothalamic tract while remaining separate (17). They terminate in the contralateral reticular formation in the brainstem, likely concentrated in the gigantocellular reticular nucleus (17, 87, 91) (Figure 3). Because of their termination in the brainstem, spinoreticular neurons are likely to be involved with both excitatory and inhibitory control of nociception due to activation of the descending modulatory system (17).

Trigeminal pathway

The spinal tract of the trigeminal nerve is located adjacent to the *fasciculus cuneatus* in the dorsal funiculus (92). It originates from the *nucleus tractus spinalis nervi trigemini* in the brainstem and extends caudally to the dorsolateral funiculi, as well as laminae I and II in the dorsal horn of the first two cervical spinal cord segments (92). This tract plays a crucial role in the transmission of orofacial pain, conveying noxious and sensory information, including touch and temperature, from the face, nasal region, oral cavity, and teeth (92, 93). A more detailed exploration of this pathway lies beyond the scope of this paper.

Nociceptive mechanisms explained

Nociception occurs when nerve cell fibers are initially activated by a harmful stimulus, which may result from physical contact (mechanical), extreme temperature (thermal), or inflammation (chemical). This process takes place in peripheral organs, including the skin, joints, or muscles as well as internal organs (94). Nociception occurs in four phases: (1) Transduction, (2) Transmission, (3) Modulation, and (4) Perception.

Transduction phase

The initial stage of nociception is the transduction phase, where nociceptors convert harmful or potentially damaging stimuli into electrical signals. Nociceptors are specialized high-threshold sensory receptors, often described as peripheral free nerve endings. However, recent findings in humans and mice suggest they may not be entirely free but are connected to specialized nociceptive Schwann cells that form mesh-like networks around the nerves and project into the epidermis alongside the nociceptors (95). Such Schwann cells have also been found in the hairy skin of cats (96). Electrical signals are transmitted from the nociceptors by peripheral afferent pseudounipolar neurons located in the dorsal root ganglion outside the spinal cord (9, 10). They are characterized by having split axons that allow them to connect inputs from the periphery to the spinal cord and contain a subset of nociceptive fibers called Aδ or C fibers. These fibers differ in various aspects, such as diameter, degree of myelination, and conduction velocity (9). Aδ fibers are small neuronal fibers (about 2–6 μm in diameter) with a thin layer of myelin, enabling them to transmit signals at high velocity (2, 97). Due to their rapid response, they are responsible for generating "the first pain," which is a short-lasting, sharp, and pricking sensation that serves as a warning sign (93, 98). Twenty percent of all cutaneous Aδ fibers respond exclusively to noxious mechanical stimulation, while others are also involved in mediating thermal nociception (11, 99). The small surface area and clustered nociceptors of Aδ fibers enable precise identification of sensations, allowing the body to accurately pinpoint the specific area under threat (100, 101). In contrast, the C fibers are smaller (about 0.4–1.2 μm in diameter) and have no myelin, resulting in slower conduction velocities (2, 97). They are responsible for "the second pain," a throbbing or burning sensation that develops slowly but lasting longer and is difficult to localize precisely due to their large surface areas (93, 98). C fibers can respond to thermal, chemical, and mechanical stimulation and make up 40–90% of the afferent fibers in a cutaneous nerve in cats (15, 99). In general, somatic tissues have a higher density of nociceptors with smaller receptive fields, whereas visceral tissues have fewer nociceptors, but larger receptive fields (11). This often makes it easier to localize somatic pain to a specific area, whereas visceral pain tends to be more diffuse (11). Certain types of C fiber nociceptors can be inactive (silent') under normal conditions but may become activated in response to specific stimuli. This activation can occur during periods of inflammation, which chemically stimulates the nociceptors. Once activated, these nociceptors can also respond to mechanical or thermal stimulation (11). Besides Aδ and C fibers, peripheral afferent neurons may also contain Aβ fibers. Aβ fibers are large low-threshold neuronal fibers (> 10 μm in diameter) that typically respond to innocuous stimuli and are rarely involved in nociception (2, 97). However, under certain conditions, these fibers may undergo a functional switch, beginning to produce Substance P (93, 102). This change is critical in the development of tactile allodynia, a condition in which normally non-painful mechanical stimulation, such as touch, triggers a painful response (93). This phenomenon can be observed in some patients with neuropathic pain (93).

Common neurotransmitters and receptors

The generation, transmission, and modulation of nociceptive signals in the dorsal horn of the spinal cord is a complex process involving the release of various neurotransmitters. These neurotransmitters can have excitatory, inhibitory, or dual functions depending on the receptors to which they bind (Table 2) (14). Most neurotransmitters are either amino acids or neuropeptides, and nociceptors can be classified into those that contain neuropeptides and those that do not (103).

Excitatory neurotransmitters, such as glutamate - the primary excitatory neurotransmitter in the central nervous system - are important for generating fast, short-lived signaling throughout the nervous system including the thalamocortical areas (15). Glutamate binds to the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and N-methyl-D-aspartate (NMDA) receptors, generating rapid action potentials that are critical for sensory processing, including nociceptive pathways (11, 14, 104). Neuropeptides, including Substance P and Calcitonin Gene-Related Peptide (CGRP), bind to neurokinin receptors, such as the neurokinin 1 (NK1)

TABLE 2 Common neurotransmitters and receptors involved in nociception and pain.

Neurotransmitter	Receptor	Function
Glutamate	AMPA NMDA Kainate	Excitatory
Substance P	Neurokinin 1 (NK1)	Excitatory
CGRP	Neurokinin receptors such as CALCRL	Excitatory
GABA	GABAA GABAB	Inhibitory
Glycine	GlyR	Inhibitory
Serotonin	5-HT	Excitatory/ inhibitory
Noradrenaline	α1 α2 β1 β2 β3	Excitatory/ inhibitory

Various excitatory and inhibitory neurotransmitters are involved in the generation of pain. Excitatory neurotransmitters such as glutamate bind to α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and N-methyl-D-aspartate (NMDA) receptors, generating rapid action potentials that are critical for sensory processing, whereas Substance P and Calcitonin Gene-Related Peptide (CGRP) bind to Neurokinin 1 (NK1) receptors, generating prolonged postsynaptic potentials to enhance NMDA receptor activity. Inhibitory neurotransmitters, including GABA and glycine, modulate sensory inputs and helps maintain a balance between excitatory and inhibitory activity in the central nervous system. AMPA: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; CALCRL: Calcitonin-receptor-like receptor; CGRP: Calcitonin Gene-Related Peptide, NK1: Neurokinin 1; NMDA: N-methyl-D-aspartate.

receptor, to generate slower, prolonged postsynaptic potentials which may reinforce the effect of the activated AMPA receptors (11). Primary afferent C-fibers release both excitatory neuropeptides and amino acids including Substance P and glutamate (105, 106). By producing these mediators, along with cytokines and other chemical signals during a painful condition, additional inflammatory cells are recruited and may participate in prolonging the sensation of pain (93).

In the dorsal horn, Substance P facilitates nociceptive transmission by binding to NK1 receptors located primarily in lamina I-III (106, 107). Other peptides, such as CGRP, can further enhance NMDA receptor activity, inducing a sensitized state that increases nociceptor sensitivity. This heightened sensitivity may contribute to the development and persistence of chronic pain conditions (16). Calcitonin Gene-Related Peptide is found in Aδ and C fibers within the dorsal root ganglion, Lissauer's tract, and the terminals of primary afferent fibers, particularly in laminae I and II (108). Alongside excitatory neurotransmitters, inhibitory neurotransmitters like GABA and glycine play a crucial role in modulating incoming sensory inputs. GABA is the primary inhibitory neurotransmitter in nociceptive processes, playing a crucial role in maintaining the balance between excitatory and inhibitory activity within the central nervous system (16, 105). In the dorsal horn, GABA receptors are widely distributed including on primary afferent terminals, interneurons and descending fibers (109, 110).

Serotonin and noradrenaline are involved in both inhibitory and excitatory processes. Serotonin primarily originates from the raphe nuclei in the brainstem, while noradrenaline is primarily sourced from the locus coeruleus in the brainstem (16). These neurotransmitters are integral components of the descending modulatory system, which regulates nociceptive processing (111–114). Serotonin and noradrenaline may also be released during normal physiological events or in response to inflammation, where they participate in lowering the nociceptive threshold (15, 104). The role of serotonin and noradrenaline as part of the descending modulatory system is discussed later in this paper.

The principle behind pain management is based on pharmacological modulation of neurotransmitters and receptors. Drugs such as gabapentin and pregabalin modulate voltage-gated calcium channels, reducing the release of glutamate and Substance P (115, 116). Maropitant, a drug that blocks the binding of Substance P to its receptor (NK1), may offer a potential treatment option for neuropathic pain although it is primarily used as an antiemetic in veterinary practice. Its analgesic effects in humans have been largely disappointing, though it has shown some variability in analgesic responses in cats and can be used to reduce phantom scratching in dogs with syringomyelia (117, 118). Tramadol, a synthetic analgesic, is metabolized in the liver into two metabolites, one acts as an opioid agonist, and the other enhances the inhibitory effects of noradrenaline and serotonin (117). Amitriptyline, a tricyclic antidepressant, is occasionally used to treat chronic neuropathic pain in animals. It has a complex pharmacological profile with actions on serotoninergic and noradrenergic systems as well as anticholinergic and antihistaminergic properties and NMDA receptor antagonism (117). Selective serotonin-norepinephrine reuptake inhibitors (SSRIs), another class of antidepressants, work by inhibiting the reuptake of serotonin and noradrenaline, thereby prolonging their effect, and potentially alleviating neuropathic pain (117).

Magnesium has been investigated as a potential therapeutic agent for pain management due to its antagonistic action at NMDA receptors, preventing the process of central sensitization and mitigating preexisting pain hypersensitivity (119). Central sensitization is discussed in greater detail in the section on modulation. As a natural calcium antagonist, magnesium may also exert its analgesic effects through calcium channel blockade, which has been identified as a therapeutic target in neuropathic pain conditions (119). In combination with NMDA antagonists such as ketamine, magnesium has been shown to enhance the drug's binding affinity and inhibit calcium influx through blockade of the NMDA receptor, suggesting a synergistic effect in pain modulation (119).

Preventative strategies may also be beneficial in reducing or preventing central sensitization. Local anaesthetic blocks can help prevent the induction of central sensitization in animals with pre-existing painful conditions undergoing procedures, such as cats with feline orofacial pain syndrome or tooth resorptions undergoing dental care (100). By incorporating targeted interventions, the progression of central sensitization may be attenuated, thereby improving pain management outcomes and chronification of pain.

Transmission phase

During the transmission phase, nociceptive signals are transported from the periphery through the spinal cord and to the brain. When peripheral afferent neurons reach the dorsal horn, the primary area for somatosensory processing in the spinal cord, they synapse with

projection neurons. Projection neurons have long axons that can extend across multiple spinal cord segments and brain regions before finally terminating in the brain (57). Projection neurons may also have collateral branches that synapse in various regions along their pathway (120, 121). Depending on the region of the dorsal horn from which they originate, projection neurons are characterized by specific neuroanatomical features, such as termination site, axon length, number of collateral branches, and the nociceptive input they transmit. In the spinal cord, nociceptive projection neurons may be activated only by nociception (nociceptive-specific), or they may respond to a range of stimuli, including light touch and different ranges of noxious stimulation (WDR neurons) (62, 63).

Nociceptive-specific neurons are typically found in the superficial laminae of the dorsal horn, while WDR neurons are located in deeper layers. However, WDR neurons may contain large dendrites that extend into the superficial layers to synapse with primary afferent fibers (98, 122).

Modulation

In 1965, Melzack and Wall introduced the gate control theory of pain to explain phenomena such as the relief of itching or pain sensation when rubbing a nearby area (107, 123). They proposed that lamina II in the dorsal horn acts as a "gate," which opens and closes to sensory and nociceptive peripheral inputs before projecting them to the brain. Essentially, this theory describes how the activation of Aβ fibers by non-noxious stimuli can disrupt the transmission of nociceptive signals by C fibers (99, 100). Although later studies have found the gate control theory limited due to the diversity of neurons involved, it significantly advanced our understanding of pain physiology and nociceptive modulation (124, 125).

Nociceptive signals undergo numerous complex modulations before reaching the brain and being perceived as pain (15, 19). Modulation designates the process in which descending projection neurons, interneurons or the brain interact with incoming noxious input in the spinal cord, enhancing or reducing the signal (15, 19, 126). This modulation involves different adaptations and reorganization in response to injury, persistent nociceptive input, or external stimuli and is referred to as nervous system plasticity or 'neuroplasticity' (19). This phenomenon occurs throughout the nervous system, including the peripheral nerves, where nociceptors may become sensitized (peripheral sensitization), the spinal cord (central sensitization), and the brain (via altered processing in pain-related cortical regions or through changes of the descending pathways) (19, 127). These alterations result from molecular changes, such as the upregulation of excitatory neurotransmitters like glutamate and the downregulation of inhibitory mediators such as GABA (19).

Spinal and supraspinal mechanisms play crucial roles in controlling nociceptive inputs to the brain (125).

Spinal modulation

In the spinal cord, interneurons play a crucial role in local modulation by directly or indirectly influencing projection neurons through the release of various neurotransmitters (126). Nitric oxide (NO) is a key signaling molecule involved in the modulation of nociception within the spinal cord. Its production is primarily triggered by calcium influx through NMDA receptors, which activates nitric oxide synthase (NOS). However, NO can also be generated in response to tissue damage and inflammation, as pro-nociceptive mediators—including hydrogen ions (H^+), potassium ions (K^+), and adenosine triphosphate (ATP)—activate NOS (14, 128–130). The involvement of NO in nociception is particularly prominent in inflammatory and neuropathic pain (14, 129). Inflammatory states lead to the release of cytokines and prostaglandins, which can induce NO production in certain neurons and glial cells within the dorsal horn of the spinal cord (128, 131). NO facilitates pronociceptive signaling by sensitizing dorsal horn neurons, enhancing excitatory neurotransmitter release from primary afferent nociceptive fibers, and promoting the synthesis of prostaglandins and cytokines (128).

Wind-up and central sensitization

Pain perception is significantly influenced by spinal cord and medullary dorsal horn neurons through a process known as "wind-up." This phenomenon is characterized by a progressive and prolonged increase in neuronal excitability following repetitive stimulation of C fibers at a constant intensity. It involves neurotransmitters such as Substance P and glutamate, which act on their respective receptors, NK1 and NMDA (132, 133). Under normal conditions, NMDA receptor channels are blocked by magnesium ions, preventing calcium influx despite glutamate binding (119, 134). However, depolarization of the post-synaptic membrane—either through Substance P activation of NK1 receptors or glutamate binding to AMPA receptors—removes this magnesium block, allowing NMDA receptor activation (119, 134). Repeated NMDA receptor activation, along with increased glutamate influx and voltage-gated calcium channel activity, triggers intracellular signaling cascades that progressively enhance neuronal firing frequency and intensity, even with low-frequency stimulation (119, 134). This process increases synaptic plasticity and enhances dorsal horn neuron excitability, making them more responsive to incoming pain signals (130, 134). Wind-up is a critical early step in the development of central sensitization.

Central sensitization is driven by key neurotransmitters, including glutamate, Substance P, and brain-derived neurotrophic factor (BDNF), which interact with receptors such as NMDA, mGluR, NK-1, and TrkB (130). These interactions elevate intracellular calcium levels and activate intracellular signaling pathways, leading to enhanced receptor function and increased receptor recruitment to the cell surface, ultimately amplifying synaptic reactivity (130). Other mechanisms contributing to central sensitization include ectopic action potential generation, facilitation and disinhibition of synaptic transmission, immune cell interactions, and structural synaptic changes (100). The result is an amplification of pain signals and heightened pain perception, which can persist long after the resolution of the initial injury or inflammation (100).

Glial cells

Another important spinal modulator are the glial cells. Glial cells, especially astrocytes and microglia, play important roles in the

development and persistence of chronic and neuropathic pain by actively modulating neuronal activity and contributing to neuroinflammation (135–137). Microglia influences plasticity and synaptic function through neuronal interactions (138). When activated, microglia can release pro-inflammatory cytokines and chemokines, promoting neuroinflammation that contribute to sustain chronic pain conditions (139).

Astrocytes are the most abundant glial cells in the central nervous system, where they support homeostasis by maintaining synaptic function, regulating and recycling neurotransmitters, and releasing other neuromodulatory substances such as growth factors (139, 140).

The activation of astrocytes and microglia in sensory pathways can enhance nociceptive signaling by increasing neuronal excitability and reduce the body's inhibitory control, which potentially may lead to increased and abnormal pain sensations through central sensitization as seen in chronic and neuropathic pain conditions (125, 141). This can occur with dorsal horn nerve injuries, where microglial activation triggers the synthesis and release of BDNF (125, 142). BDNF reduces GABAergic inhibition, which paradoxically can result in excitatory effects – a phenomenon called disinhibition, which leads to further sensitization and neuroplastic changes in the pathway (125, 142). Pathological activation of astrocytes can exacerbate neuroinflammation, inhibit axonal growth, and suppress synapse formation, thereby disrupting sensory neural circuitry (136).

In the dorsal root ganglion, satellite glial cells amplify nociceptive signals and sustain inflammation by releasing growth factors and chemokines that attract immune cells (143).

Supraspinal modulation

Nociceptive signals are also modulated by supraspinal structures. The descending modulatory system, which regulates nociception at the level of the spinal dorsal horn, involves three key components: the rostral ventromedial medulla (an important source of the neurotransmitter serotonin), located on the floor of the medulla oblongata; the periaqueductal gray, a cluster of neuronal cell bodies surrounding the mesencephalic cerebral aqueduct (containing multiple neurotransmitters including glutamate, GABA, opioids particularly enkephalin, Substance P, neurotensin and endocannabinoids); and the nucleus locus coeruleus, situated in the pons (source of noradrenergic neurotransmitters) (15). These structures influence spinal projection neurons either pre- or postsynaptically, thereby modulating the transmission of nociceptive signals to the brain (18). Studies have shown that up to 50% of projection neurons in laminae I and V of the lumbar spinal cord interact directly with enkephalin terminals, an endogenous opioid with pain-suppressing effects suggesting the presence of a post-synaptic endogenous pain regulation mechanism in the feline spinal cord (85, 99). This is most likely also the case in dogs, where the distribution of enkephalins in the brain is largely similar to cats (144).

The periaqueductal gray receives input from both higher and lower structures within the central nervous system and can respond by releasing a variety of endogenous opioids, including enkephalin to modulate the inputs (59, 144–146). Additionally, the periaqueductal gray can activate other components of the descending modulatory system, such as the raphe nuclei located in the rostral ventromedial medulla (11, 114). The raphe nuclei are a critical source of serotonin and play a role in serotonergic modulation within the spinal cord (11). The analgesic effect from activation of the raphe nuclei has also been shown to involve enkephalin (147). Furthermore, the raphe nuclei can stimulate the locus coeruleus, which is the primary source of noradrenaline in the body. Noradrenaline binds to α2-receptors in the spinal cord, producing an analgesic effect (15). This mechanism is mimicked when α2 agonists are used during anaesthesia (15). Both serotonin and noradrenaline inhibit the transmission of ascending nociceptive signals from the spinal cord by activating interneurons and blocking nociceptive inputs from all layers of the dorsal horn (111–114). In mice, nociceptive neurons in lamina V-VIII project back to nucleus raphe magnus, which is part of the raphe nuclei complex. This establishes a nociceptive processing-loop between the deeper dorsal horn and the raphe nuclei (148). In cats, serotonin binding follows a laminar distribution in the spinal cord, with high binding levels in laminae II and III and reduced binding in the deeper dorsal horn laminae, except for lamina I, which also shows low binding (113). Dogs exhibit a similar pattern, where the density of serotonin fibers decreases ventrally through the dorsal horn, with low-level expression in lamina II (149). The clinical effect of this species-specific difference is unknown to the authors. Additionally, the thoracolumbar spinal cord is richer in serotonin fibers than the cervical cord (149). These serotonin fibers travel craniocaudally through the white matter funiculi, sending horizontal branches into the gray matter and extending to the tail and *filum terminale* (149).

In human chronic neuropathic pain conditions, the phenotype of the descending inhibitory pathway may shift from being predominantly controlled by noradrenergic and serotonergic mechanisms to involving other neurotransmitters (63). Additionally, this pathway can become depleted, leading to reduced opioid receptor function (93). Since opioids play a crucial role in the descending inhibitory pathway, this impairment results in increased neuronal firing at the spinal cord level, ultimately amplifying pain perception (93).

The role of the thalamus in nociceptive transmission and pain generation

Before reaching higher brain structures and being perceived as pain, most nociceptive signals pass through the thalamus. The thalamus is a gray matter structure composed of several nuclei located in diencephalon that processes and distributes most of the sensory inputs coming from the body or higher brain structures. The thalamus in cats and dogs is quite similar and contains 18 nuclei grouped together (150). It is divided into external and internal laminae, which are further organized based on their anatomical orientation (92, 150). The intralaminar nuclei, which are activated by axons from the reticular formation, are grouped into medial and lateral categories (92, 150). When activated from nociceptive inputs, the medial thalamic nuclei help generate emotional and motivational aspects of pain due to its connection to the limbic system (15). In contrast, lateral nuclei are involved in the sensory-discriminative aspects of pain, projecting to the somatosensory cortex (15). Both groups are further divided into dorsocaudal and ventral nuclei (92). The ventral nuclei are key for transmitting nociceptive signals and include subgroups like ventral rostral, lateral, caudal, and medial nuclei (92, 150). Specifically, the ventral caudal-lateral nucleus (*nucleus ventralis caudalis*) relays pain signals to the somatosensory cortex (92) (Figure 4).

Perception

Conscious pain perception arises from the activation of multiple brain structures collectively referred to as the pain matrix. This term describes a theoretical cortical network involved in pain perception, integration, and modulation. The pain matrix includes the amygdala, hippocampus, somatosensory cortex, insular cortex, cingulate cortex, prefrontal cortex, and regions within the frontal and parietal lobes (100).

This network integrates emotional and cognitive processes, generating both emotional and behavioral responses to pain.

Pain perception within the pain matrix can be categorized into three main components:

1. Sensory-discriminative – responsible for the localization, intensity, and duration of pain.
2. Affective-motivational – related to the emotional response to pain.
3. Cognitive – governing the behavioral and evaluative aspects of pain

The primary and secondary somatosensory cortices (S1 and S2) play a crucial role in the sensory-discriminative aspect of pain, encoding information about its location, intensity, and duration (100).

However, pain perception is influenced not only by the sensory registration of nociceptive signals but also by various other factors, including environment, past experiences, cognitive function, and emotional state (100, 151). Just as lower components of the pain pathway undergo modulation, the brain itself can also adapt, potentially altering pain perception (152). Central sensitization within the dorsal horn can amplify and enhance nociceptive inputs reaching the brain, intensifying pain perception (19). Under normal conditions, neuroplasticity supports healing and adaptation. However, in chronic pain conditions, prolonged exposure to heightened nociceptive input can lead to maladaptive changes in brain regions associated with pain processing (6, 153, 154).

These changes often involve increased activity in areas linked to the emotional and cognitive aspects of pain, such as the rostral (anterior) cingulate cortex and insula (154, 155). This highlights the importance of a comprehensive pain management approach, particularly when conventional treatments fail to achieve the desired outcomes. Effective pain management should address not only the sensory aspects of pain but also the maladaptive neural changes and the cognitive and emotional factors that shape the pain experience.

Cognitive component of pain

The cranial cingulate cortex, in addition to the prefrontal cortex, plays a role in advanced cognitive behavior, decision-making, and expression of both personality and social behavior (100). In cats, a direct projection from the ventral area of the thalamus, including the ventral caudal-lateral nucleus, to the cingulate cortex has been identified, providing a direct connection between nociceptive inputs and cognitive behavior (156). A recent study found decreased volume of the cingulate gyrus in Cavalier King Charles Spaniels with Chiari-like malformations and syringomyelia, supporting the hypothesis that chronic pain can also change the overall function of the central nervous system in the dog brain (153). Pain-induced

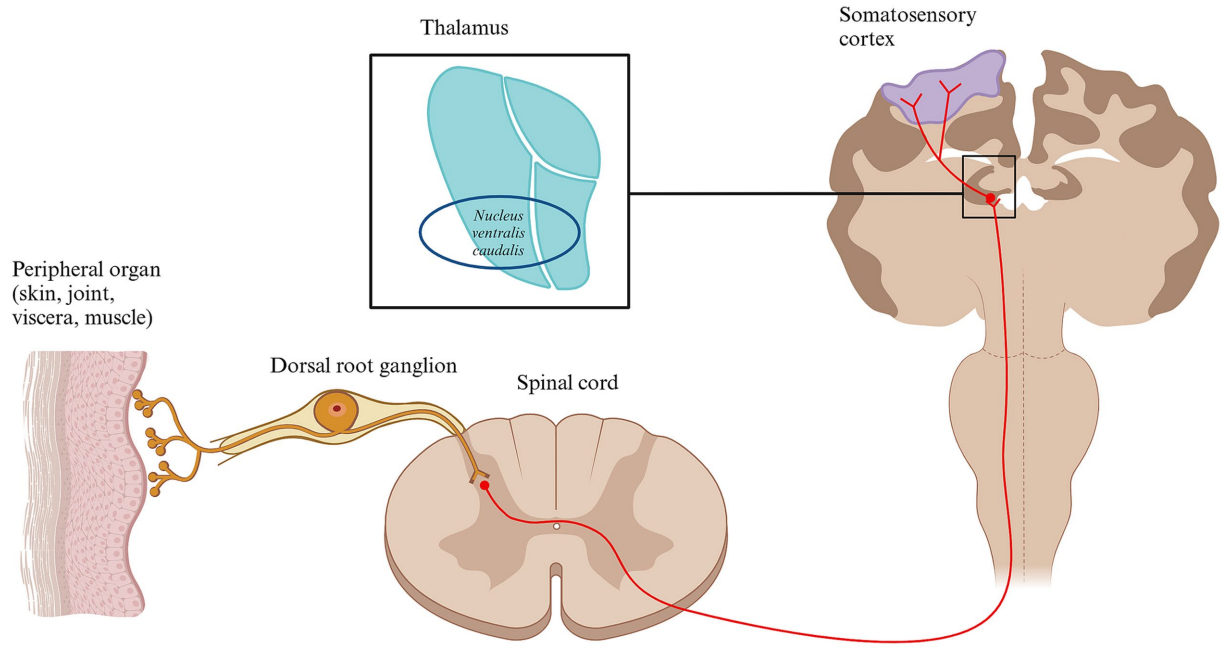

FIGURE 4
Schematic diagram of the thalamus and the somatosensory cortex. Conscious pain perception is caused by the activation of several brain structures. Following transmission through different nociceptive pathways, most nociceptive inputs reach the thalamus. One of the most important thalamic nucleus for processing nociceptive input is the ventral caudal-lateral nucleus. From here, the nociceptive signals are distributed to multiple higher brain areas, including the primary and secondary somatosensory cortex, contributing to the subjective perception of pain. Other relevant brain structures involve amygdala, the insula and the cingulate cortex. Created in BioRender. Pedersen (2025) https://BioRender.com/t93x590.

changes to the cingulate cortex may therefore affect cognitive or emotional functions. This has recently been demonstrated in a study reporting that chronic musculoskeletal pain in working dogs may affect cognitive function by impairing spatial working memory (157).

Emotional component of pain

Although pain-related effects on emotions have not been well studied in animals, they are well documented in humans with chronic pain. People suffering from chronic pain often experience depression and anxiety along with higher degrees of frustration (158). This is believed to arise from an increased activation of the limbic system by nociceptive input. The limbic system contains regions involved in emotions, feelings, and memory. In pain perception, the limbic system helps generate memories and behaviors that serve to protect and prevent the individual from a future painful situation. In particular the amygdala and hippocampus are important for the generation and consolidation of pain-related memories, serving the purpose of preventing the animal from encountering a similar situation in the future. However, together with the prefrontal cortex the amygdala and hippocampus can contribute to a negative emotional state and anxiety triggering the release of adrenaline following a painful stimulation (100). Similarly, the periaqueductal gray not only processes nociceptive information, but also processes other sensory inputs that are relevant for regulating emotional motor behaviors, including defensive responses such as fighting or flight (59, 60, 159). The lateral periaqueductal gray mediates autonomic hypertension, whereas the caudolateral region is associated with flight behavior. Studies in cats have shown that spinocervicothalamic and spinomesencephalic neurons project to these areas, suggesting that nociception mediated by these nociceptive tracts may mediate hypertension and behavior associated with anxiety (60–62). Nociceptive-induced activation of these brain structures creates a strong relationship among negative emotional states, anxiety, and pain that should not be forgotten in animals. A recent study showed that fear and anxiety, as well as the dog's ability to handle novel or challenging situations and engage socially, may be predictors of chronic pain (160). Dogs with chronic musculoskeletal pain had a higher frequency of fear and aggression and had poorer coping ability with daily changes (160). Similarly, chronic stress, such as social conflict, can exacerbate pain conditions, as observed in cats with feline orofacial pain syndrome. This

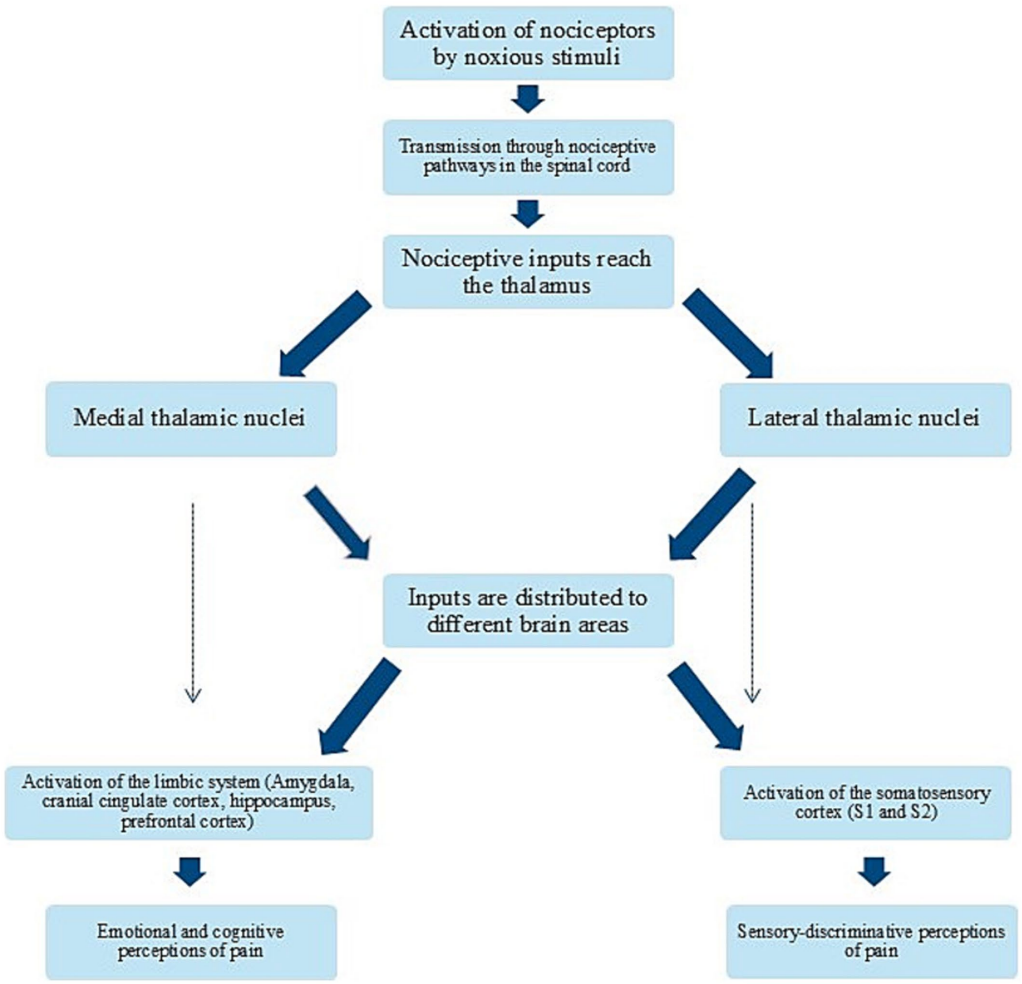

FIGURE 5
Flow chart of the process from nociception to pain perception. Simplified flow chart illustrating the process from nociceptor activation to conscious perception of pain and its consequences.

highlights a bidirectional relationship between stress, anxiety, and pain, where stress amplifies pain perception, and persistent pain, in turn, increases stress and anxiety (100). Thus, the synchronous activation of multiple brainstem and higher brain structures are contributing to the full pain experience (Figure 5). It is therefore important to consider pain not only a sensory-discriminative experience, but also an emotional experience and promote positive emotions as a multimodal component when treating veterinary patients with pain.

Limitations of this review

The field of pain research is vast, encompassing diverse topics from studies on ion channels and intracellular structures to investigations using rodent models and clinical studies in large mammals, primarily humans. This review aims to provide a broad overview tailored for veterinary clinicians and does not attempt to be exhaustive. Readers seeking more detailed or comprehensive insights are encouraged to consult specialized journals and reviews dedicated exclusively to pain research.

Conclusion

A thorough understanding of pain processes and pathways is essential for veterinary professionals to deliver effective pain management for canine and feline patients. This review highlights key aspects of spinal nociception and pain in dogs and cats. While significant advancements have been made in understanding nociception and pain pathways in these species, further research is needed, particularly on biomarkers of chronic pain, methods of assessing and monitoring animals with chronic pain and species-specific mechanisms of cognitive and emotional pain perception in chronic pain conditions.

Author contributions

TP: Conceptualization, Visualization, Writing – original draft, Writing – review & editing. MB: Conceptualization, Supervision, Writing – review & editing. CR: Supervision, Visualization, Writing – review & editing.

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Conflict of interest

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Lumbosacral (myelo) meningoceles in dogs, related tethered cord syndrome, and their surgical management: review of the literature and clinical experience

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Neural tube defects (NTDs) are a group of congenital malformations characterized by various levels of protrusions of meninges with or without nervous tissue through incomplete osseous coverage (cranium bifidum for the cranial forms and spina bifida for spinal meningoceles/myelomeningoceles [MCs/MMCs]), with associated dorsal midline cutaneous signs. Amongst a confusing vocabulary, spina bifida is both the term most used to refer to NTDs and the most common manifestation of NTDs, with a predilection for the lumbosacral area in screw-tail breeds. With the growing popularity of bulldogs, lumbosacral (LS) MCs/MMCs are increasingly encountered, and small animal practitioners should learn to recognize them. Clinical signs may include urinary and/or fecal incontinence, pelvic limb neurological deficits with bunny hopping (neurolocalization L4-caudal or subset), and cutaneous signs (swirl of hair and dimple); the combination of which is pathognomonic of these disorders in bulldog puppies. Since these malformations often trigger a tethered cord syndrome (TCS), neurological worsening is possible. While historically reported to be somewhat hopeless regarding neurological improvement, isolated case reports, small case series, and personal experience of the author indicates that post-operative improvement is possible. Review of the literature (14 cases) and personal surgical experience (9 cases) retrieved 23 canine cases of LS MC/MMC treated surgically with follow-up. Clinical presentation, diagnostic imaging findings (CT and MRI), and intra- and post-operative findings are discussed in this article, along with a detailed description of the surgical technique. Pelvic limb deficits improve post-surgically in most cases (14/17 [82%] cases with pre-operative deficits and follow-up ≥1 month) albeit sometimes only marginally. Urinary/fecal continence can improve also, although less frequently (10/21 [48%] at 1 month follow-up and 8/21 [38%] at ≥6 months).

KEYWORDS

meningocele and meningomyelocele, spina bifida (SB), myelomeningocele (MMC), tethered cord syndrome (TCS), surgery, neural tube defect (NTD), neural tube defect management, Bulldogs

Introduction

Meningoceles and *myelomeningoceles* (MCs/MMCs) are specific types of *Neural Tube Defects* (NTDs), which are characterized by failure of differentiation, formation, and separation of the *neural tube* (NT) from the rest of the ectoderm during neurulation. Dorsal midline cutaneous signs ensue frequently, albeit sometimes subtle, such as the "hair collar" sign in humans with mild cranial forms (1, 2). NTDs have been reported as far back in time as the Neolithic period in humans (3), with representations of such malformations in early civilizations through pottery, sculptures, and other figures (4, 5). As an example, the Olmec (Mesoamerican pre-Columbian civilization) motif of the Were-Jaguar and other figurines representing shamans with specific social status are at least partially inspired by midline defects and physical characteristics of NTDs and MMCs, such as cleft foreheads, cleft/bifid noses, hunchbacks, lumbar kyphosis, sitting positions (due to paralysis of the legs) and circular areas of different skin color in the lower back (representing the cutaneous manifestation of lumbosacral NTDs) (4, 5) (Figure 1).

The first extensive, illustrated description of lumbosacral (LS) MC/MMC and surgical correction, along with the first use of the term *spina bifida* (SB), must be credited to the Dutch surgeon Nicolaes Tulp (1593–1674) in his book *Observationes medicae* (6). In humans, MC/MMC is a clear indication for surgery, with the current standard of care being in-utero surgery since the findings of the MOMS trial in 2011 showing clear benefits of prenatal vs. postnatal surgical correction (7). Although reports of MCs/MMCs exist in the veterinary literature for more than a century, they are most commonly descriptive, and there is currently a lack of long-term clinical studies evaluating surgical or medical management in companion animals. NTDs such as MCs/MMCs are most often encountered in the LS area in screw-tail breeds (e.g., Bulldogs for dogs, Manx for cats), but have also been reported in other breeds and other areas along dorsal midline of the neuraxis (e.g., head, cervical and thoracolumbar vertebral column) (8–11) (Figure 2). For LS MCs/MMCs, the classical presentation is urinary and fecal incontinence with neurological deficits of variable severity affecting the pelvic limbs in a young/immature animal. Although historically reported to be static clinically, these disorders can be progressive if associated with *tethered cord syndrome* (TCS) and the neurological deficits observed can worsen in the absence of surgical correction. As Bulldogs have become some of the most popular breeds and owners reluctant to euthanize, patients with LS MCs/MMCs are seen more frequently in need of a therapeutic option.

Through previous work with Bulldog rescue organizations, the authors were involved with multiple cases of LS MCs/MMCs, including 9 cases treated surgically, some previously reported (12). In the English veterinary literature, the authors could retrieve 14 canine cases of LS MC/MMC treated surgically with information regarding clinical signs and outcome (albeit occasionally incomplete) (13–19). Two other Bulldogs were treated surgically for LS MC/MMC with overall similar technique, but the procedure included intra-operative application of allogeneic placenta-derived mesenchymal stem/stromal cells at the surgical site (20), so they are not included in the results of this review. This article attempts to clarify the terminology and concepts of NTDs before reviewing the literature and personal experience of the authors with surgical management of canine LS MCs/MMCs.

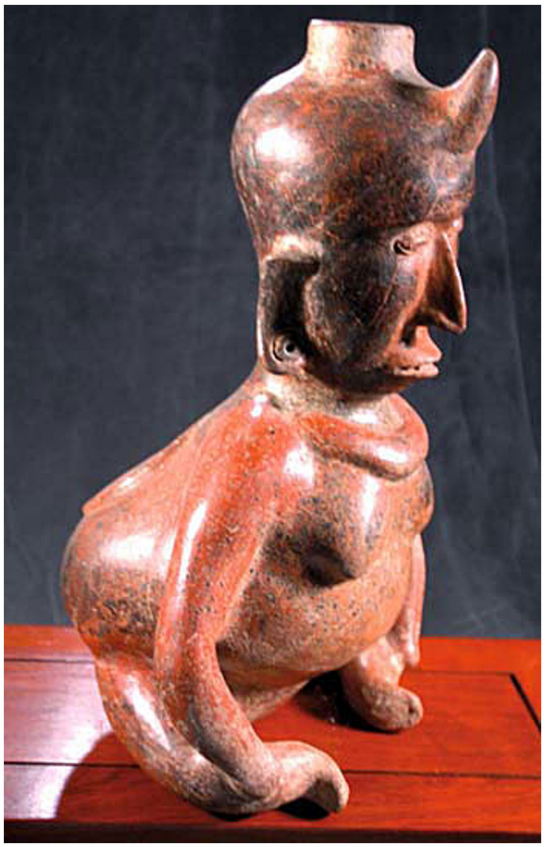

FIGURE 1
Shaman figure typical of pre-Columbian cultures (Olmec, Nayarit, Colima), West Mexico (circa 200–400 AD). Note the distinctive features of neural tube defects (NTDs) on midline: the "horn," indicating the shamanic status of the individual, is likely a representation of encephalocele/persistent bregmatic fontanelle associated with this large hydrocephalic head, while a circular medallion is represented on lumbar dorsal midline, where lumbosacral meningoceles/myelomeningoceles (LS MCs/MMCs) show birthmarks or placodes of neuroectodermal tissue. The classic forward-leaning position and severe lumbosacral kyphosis indicate the muscle weakness and vertebral column deformation common with NTDs, while the atrophied legs and their position represent the orthopedic anomalies and paraplegia associated with a LS MMC and severe tethered cord syndrome (TCS) [from Goodrich, J.T. (4), with permission from Springer Nature].

Terminology

> *"Ce que l'on conçoit bien s'énonce clairement…"* (what we understand well we explain clearly [Nicolas Boileau 1636–1711]).

The terminology used in the medical literature on NTDs is confusing to say the least. Terms employed are somewhat redundant (e.g., MCs/MMCs are exemples of NTDs, myelodysplasia, spinal dysraphisms and also imply the presence of spina bifida) and confusing due to the subtlety in their differences or lack thereof (e.g., myelodysplasia defined as "any malformation of the spinal cord

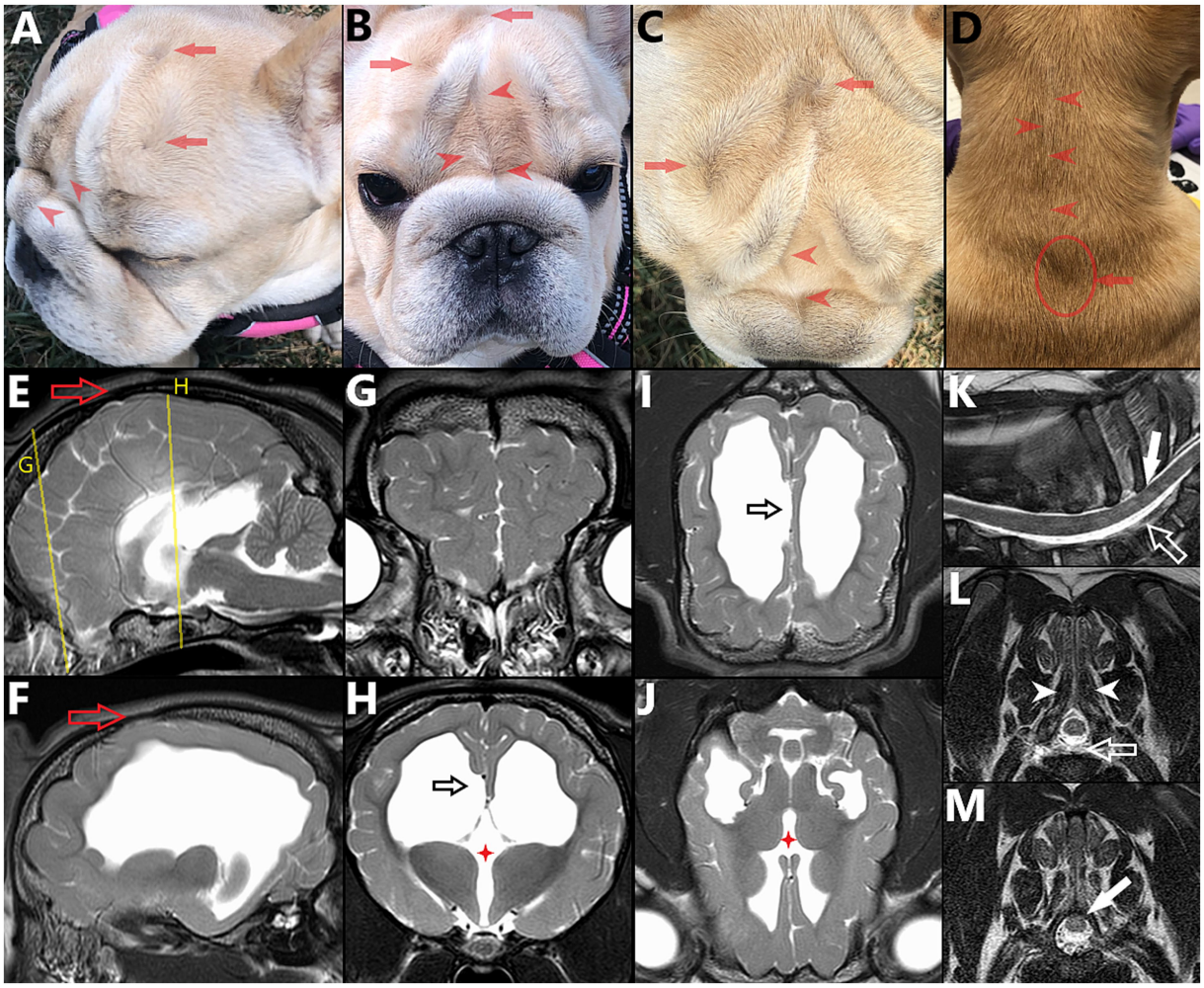

FIGURE 2
6 month-old female French Bulldog illustrating the concepts of multiple sites of neural tube defects (NTDs) and external/cutaneous signs (visible on dorsal midline) being suggestive of NTDs. **(A)** Lateral, **(B)** frontal, **(C)** dorsal views of the head and **(D)** dorsal view of the neck, with corresponding magnetic resonance images (MRI) vertically aligned. **(E)** Midline and **(F)** para-midline sagittal T2W images of the brain, indicating the level of the transverse T2W images of the **(G)** frontal lobes and **(H)** thalamus, with dorsal T2W images at the level of the **(I)** corpus callosum and **(J)** thalamus. Note the bifid nasal planum (complete cleft in **B**), change of hair implantation with a ridge of hair in the frontal area and on dorsal cervical midline (*red arrowheads* in **A–D**) and swirls of hair over the calvaria (reminiscent of the "hair collar" sign in humans) (*red arrows* in **A–D**), associated with palpable osseous defect (cranium bifidum occultum, *red hollow arrow* in **E,F**). Features of NTD affecting the brain are visible, including hydrocephalus **(F,H–J)**, abnormal gyrification of the frontal lobes **(G)** and failure of interhemispheric midline fusion with agenesis/dysgenesis of midline structures: interthalamic adhesion (*red four points star* in **H,J**) and corpus callosum (*black hollow arrow* in **H,I**, note the abnormal folding of the cingulate gyrus). In the cervical area, under the less pronounced swirl of hair (seen in **D**), **(K)** sagittal and transverse T2W MR images at **(L)** caudal aspect of T1 and **(M)** mid-T2 show further features of NTD/spina bifida: only partially fused spinous process at T1 (*white arrowheads* in **L**), dorsal deviation of the dural sac within the vertebral canal (*white hollow arrow* in **K,L**) and dorsal deviation of the spinal cord within the dural sac (with obliteration of the dorsal subarachnoid space), tucked under the lamina of T2 (*plain white arrow* in **K,M**).

owing to abnormal interaction of the notochord, paraxial mesoderm, and neural plate during neurulation" and dysraphism defined as "failure of neural folds to oppose and close, resulting in failed neural tube closure") (21–23). Proper terminology is also inconsistently or inaccurately applied (the terms NTDs, myelodysplasia, spinal dysraphism and spina bifida are often used interchangeably, while stricter definitions differentiate them; also see Discussion on dermoid sinus in the veterinary literature), and with wide interspecies meaning variation (e.g., while in humans the term MMC is used for NTDs with protrusion to the surface of a placode of abnormal neuroectodermal tissue (Figure 3), the protruded meninges are most commonly covered by the skin and various levels of fibrous tissue in dogs – more similar to human lipomyelomeningoceles which are skin covered NTDs). Table 1 presents a classification and the terminology associated with NTDs, along with the associated bone defects (cranial and spinal malformations are presented, respectively, on the left half and right half of the table, at similar level of severity). Spina bifida, although referring strictly speaking only to the bony anomaly, remains the term most used to designate these NTDs, likely due to historical ability to diagnose it on radiographs. Naming the underlying CNS malformation is more descriptive, useful clinically (spina bifida is not, as the bony malformation is clinically irrelevant from a neurological perspective) and should be preferred when discussing these NTDs.

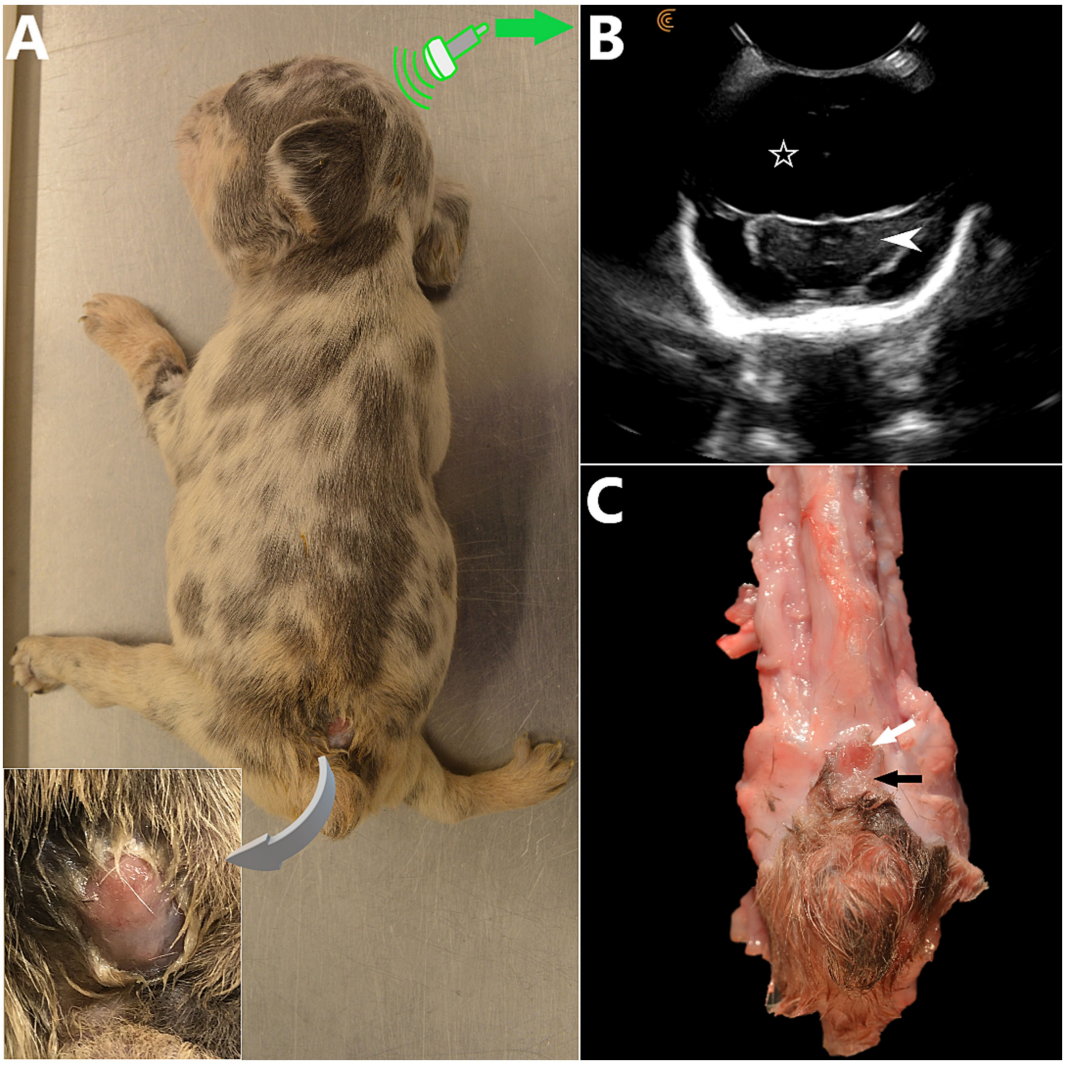

FIGURE 3
(A) 5 week-old female French Bulldog with severe, open lumbosacral myelomeningocele (LS MMC) and spina bifida (SB) at S1-S2 with protrusion of a placode of abnormal, non-neurulated neuroectodermal tissue in the LS area (similar to human MMCs). Two other dogs from the litter were euthanized due to severe malformations at delivery (C-section). This puppy had severe neurological deficits and orthopedic malformations (non-ambulatory tetraparesis markedly worse in the malformed pelvic limbs), dome-shaped head and persistent bregmatic fontanelle. The placode of non-neurulated neuroectodermal tissue is covered with a layer of dysplastic epidermal tissue, continuous with it and ulcerated segmentally. CSF leakage was present at this site (*insert*). **(B)** Transfontanelle brain sonogram, transverse view at the level of the diencephalon (*white arrowhead*) showing hydrocephalus with severely enlarged anechoic lateral ventricles (*white hollow star*). **(C)** Anatomical specimen, spinal cord removed (cranial aspect at the top). The non-neurulated spinal cord is visible at skin level as 2 unfused hemicords (*white arrow*), bordered by an alopecic area of abnormal tissue (pial layer of the meninges having failed to close and fused laterally to the epidermis) (*black arrow*). *(A and C courtesy of Dr. Kate Sarkan).*

Etiology (pathogenesis) and pathophysiology

Embryology - neurulation

Embryologically, the spinal cord originates from the neurectoderm, after formation of the neural tube and separation from the remaining ectoderm during neurulation (24). During embryogenesis, the development of the nervous system occurs through *neurulation*, which starts by the end of *gastrulation*, when the three germinal/embryonic layers are formed (*ectoderm*, *mesoderm*, and *endoderm*). Neurulation proceeds as *primary neurulation* for the anterior and largest part of the central nervous system, and *secondary neurulation* for the most caudal part (sacral and caudal spinal segments). The formation of the neural tube occurs through infolding of the neural plate under the effect of the notochord ventrally, initial closure at (a) given point(s) of the neuraxis, and propagation in a zipper-like fashion along the long axis (Figure 4). Most commonly initial closure(s) occur(s) at some level of the brain or cervical area with a short rostral propagation leading to the *cranial neuropore* (first opening to close) and a longer caudal propagation leading to the *caudal neuropore*. Closure of the caudal neuropore marks the end of primary neurulation. Secondary neurulation is species-specific and occurs at the caudal bud/tail bud, forming the last sacral and caudal spinal segments, along with mesodermal derivatives.

Neural tube defects (NTDs) refer to different congenital anomalies characterized by failure of closure of the neural tube during neurulation.

TABLE 1 Classification and terminology of neural tube defects (NTDs).

Black arrows ↙↘ indicate subdivisions within one group of malformations; ⇔ indicate association of a nervous tissue malformation with the corresponding osseous malformation. The name of each malformation/disease is indicated in **bold**, followed by the etymology in *italic* for unusual terms, and a description of the malformation. The cranial and spinal NTDs are presented at similar level of progressive severity from top to bottom (until *craniorachischisis*). *Craniorachischisis* and *Dermoid sinus* are presented across Cranial and Spinal since they affect both.

Midline Defects Congenital anomalies occurring along the midline sagittal axis of the animal (includes Neural Tube Defects, visceral malformations such as ventricular septal defects, umbilical/abdominal hernias, gastroschisis, and external malformations such as cleft nose/palate, among others)			
Neural tube defects (NTDs) Congenital malformations resulting from failure of the neural tube to form and detach properly from the superficial ectoderm during neurulation			**Other malformations (e.g. congenital heart defects)**
Cranial NTDs		**Spinal NTDs**	
Nervous tissue anomaly	**Associated osseous defect**	**Nervous tissue anomaly**	**Associated osseous defect**
no equivalent to the term 'myelodysplasia' for cranial structures		**Myelodysplasia*** *from ancient Greek μυελός (muelós, "marrow") or myelo- (spinal cord), δυσ- (dys-, "bad" or "difficult") and πλάσις (plasis "formation").* Malformations of the spinal cord due to abnormal development of the neural plate and interaction with superficial ectoderm, mesoderm, and notochord during neurulation (includes NTDs and others such as neurenteric cysts)	
Cranial dysraphism(s) *from ancient Greek δυσ- (dys-, "bad" or "difficult") and ῥᾰφή (rhaphḗ, "seam/suture").* Malformations of the cranial neural tube and associated midline raphe mesodermal and ectodermal structures (e.g. skull, skin) (include encephaloceles, anencephaly and other midline skull defects)		**Spinal dysraphism(s)** *from ancient Greek δυσ- (dys-, "bad" or "difficult") and ῥᾰφή (rhaphḗ, "seam/suture").* Umbrella term used for malformations of the spinal cord (failure of the neural folds to close) and associated midline raphe mesodermal and ectodermal structures (e.g. vertebrae, skin); implies *NTD* or *SCM* – can be qualified of *aperta* (open – visible externally) or *occulta* (hidden)	
Dermoid/Dermal sinus Congenital malformation (NTD) due to failed separation of the neural tube from the surface ectoderm and dermal elements, resulting in tubular sacs lined with hair follicles, sweat & sebaceous glands, extending from the dorsal midline (or parasagittal for cranial locations) to various depth of underlying tissues			
Meningocele (cranial) NTD characterized by protrusion of the meninges through an open/incompletely closed cranium	**Cranium bifidum** *from ancient Greek κρανιον (kranion, "skull") and latin bifidus ("split in two").* Refers to the congenital bone defect of parietal bones in cranial NTDs (failure of midline fusion) and qualified of *occultum* if no extrusion of intracranial tissue is seen, or *cysticum* if concurrent herniation of intracranial content through the bone defect (sometimes used interchangeably with *encephalocele*)	**Meningocele (MC) (spinal)** NTD characterized by protrusion of the meninges through an open vertebral arch (spinal MC)	**Spina bifida (SB)** *from Latin bifidus ("split in two").* Refers to the congenital bone defect in spinal NTDs with failure of fusion of the vertebra(e) on dorsal midline and unfused spinous process(es) (occasionally wrongfully used to designate any spinal NTD)
Encephalomeningocele/Encephalocele cranial MC with presence of brain tissue in the protruded dural sac		**Myelomeningocele (MMC)** spinal MC with presence of nervous tissue (spinal cord/nerve roots) in the protruded dural sac	
		Lipo(myelo)meningocele spinal MC or MMC with lipomatous mass (due to mesenchymal cells entering the neural tube before closure) anchoring on the spinal cord, crossing the dural sac through a meningeal defect and connecting to dermal tissues	
Anencephaly *from ancient Greek ἀν- (an-, privative a), ἐν- (en-, "in") and κεφᾰλή (kephalḗ, "head").* Cranial form of NTD due to failure of closure of the rostral neuropore, with concurrent *cranioschisis* or *acrania* and exposure of the malformed brain to the amniotic fluid/air, resulting in erosion of the telencephalon – stillborn/lethal	**Cranioschisis** *from ancient Greek κρανιον (kranion, "skull") and σχίσις (schisis, "split").* Cranial form of NTD with failed closure of the skull, occasionally used to refer solely to the bony defect but more specific definition implies concurrent *anencephaly*	**Myeloschisis** *from ancient Greek σχίσις (schisis, "split").* Severe, open form of NTD with persistent attachment of the cutaneous ectoderm to the neural plate, resulting in the protrusion to the open air (without meningeal covering) of a flattened, plate-like ending of the spinal cord (*placode*) through a *rachischisis* – often lethal	**Rachischisis** *from ancient Greek ῥάχις (rhachis, "spine") and σχίσις (schisis, "split").* Splitform of NTD with failed closure of the vertebral arch over several consecutive vertebrae, occasionally used to refer solely to the bony malformation (regardless of concurrent nervous system malformation) but more specific definition implies concurrent *myeloschisis*
Exencephaly *from ancient Greek ἐκ- (ek-, "out of"), ἐν- (en-, "in") and κεφᾰλή (kephalḗ, "head").* Cranial form of NTD similar to anencephaly but without (yet) degeneration of the brain tissue; usually an intermediate stage and progresses to anencephaly – stillborn/lethal			

TABLE 1 (Continued)

Craniorachischisis Most severe form of NTD with concurrent features of *cranioschisis* and *rachischisis*, resulting in exposure and malformation (incomplete closure) of both brain and spinal cord – stillborn/lethal	
	Placode a thickened plate-like area of embryonic ectoderm that develops into specific adult structure (e.g. hair follicles, ears, nose); neurogenic placodes develop into nervous tissue; neural placode refers to the protruded abnormal neuroectodermal tissue in cases of *myeloschisis* and certain *MMCs*
Hydrocephalus can be encountered with cranial NTDs.	**Hydromyelia** refers to dilation/widening of the central canal, has been described in cases of spinal NTDs and may be reflective of the embryogenesis of NTDs. **Syringomyelia** refers to the formation of a syrinx in the spinal parenchyma, without communication with the central canal, hence more likely to be a consequence of the pathophysiology of NTDs (e.g. due to tethered cord syndrome) than reflective of the embryologic malformation.
Schizencephaly, from ancient Greek σχίσις (schisis, "split"), ἐν- (en-, "in") and κεφᾰλή (kephalḗ, "head"), sometimes referred to as "split-brain malformation", is a congenital malformation characterized by full thickness cleft in one or both hemispheres (from the lateral ventricles to the cortical subarachnoid space); it is not a neural tube defect	**Diastematomyelia/Diastomyelia**** *from ancient Greek διαστεμα (diastema, "cleft") and μυελός (muelós, "marrow").* One of two types of double cord malformations in which each hemicord is in its own separate dural sac but contains only lateral and no paramedian nerve roots, with a midline osseocartilaginous spur separating the two. **Diplomyelia**** *from ancient Greek διπλοῦς (diploûs, "double") and μυελός (muelós, "marrow").* Refers to double cords residing in a single dural sac with no or only fibrous midline elements. Each component half is a complete cord and therefore possesses paramedian nerve roots. **Split cord malformations (SCM, types I and II)**** A newer classification suggests the term SCM, with type I referring to 2 distinct dural sacs separated by an osseocartilagineous septum, while type 2 refers to 2 hemicords in a common dural sac, with or without a fibrous midline partial septum.

**Myelodysplasia (or Myelodysplastic Syndrome) is also used to refer to a group of cancerous conditions involving immature blood cells in the bone marrow (human medicine)*

***It is not clear that split cord malformations (SCMs) should be considered NTDs as the latter are defined with specific emphasis on the formation of the neural tube and detachment from the superficial ectoderm, not synonymous with "any neurulation-related malformation". Although evidence exists of the overlap of time of onset of SCMs and concurrent neural tube defects (e.g. lipomyelomeningocele),[a] the current main theory places the embryological origin of SCMs at gastrulation, with an adhesion between developing endoderm and ectoderm resulting in an endomesenchymal tract bisecting the neural placode and underlying notochord before neurulation, which proceeds afterwards in both hemicords.[b]*

References for Table 1 and associated endnotes are presented in Supplementary material.

Failure of closure of the rostral neuropore can lead to anencephaly, a condition characterized by lack of formation of the cerebral hemispheres (brainstem and cerebellum are abnormal, but present) and protrusion of the malformed brain through an opening in the cranial vault having failed to close (*cranioschisis*), often fatal and associated with stillbirth. Less severe forms of cranial NTDs are "closed" and compatible with life, such as encephaloceles, characterized by protrusion of the meninges with (*meningoencephalocele*) or without (cranial *meningocele*) brain tissue through a skull defect (*cranium bifidum*) that is covered with skin. Milder forms of human cranial NTDs may be suspected based on dorsal midline cutaneous signs, marks of NTDs on the ectoderm (e.g., the "hair collar sign," a ring of darker, coarser hair surrounding a congenital scalp lesion), and similar semiology can be applied to canine patients (see Figure 2). In NTDs affecting the spinal cord, the failure of the neural tube to detach from the ectoderm prevents the mesodermal structures such as the sclerotomes from surrounding the nervous tissue and prevent fusion of said bilaterally paired hemi-structures on dorsal midline (see Figure 4D). This results in dorsally opened vertebrae with 2 dorsolateral hemi-laminae and 2 unfused spinous processes, hence the term *spina bifida* (from Latin *bifidus* which means split in half by a longitudinal cleft). When the caudal neuropore is affected, this results in LS MCs/MMCs, with various levels of protrusions of the meninges +/− malformed nervous system through an open vertebral arch (potentially with protrusion at the air of malformed nervous tissue itself) in association with lack of complete midline fusion of overlying mesodermal structures (e.g., muscles, vertebral spinous process) in the lumbosacral area (see Figure 3). Open MC/MMC is a term used for cases with communication of the meninges with the skin and leakage of CSF through the integument (Figure 5).

MCs/MMCs are responsible for neurological deficits due to the anatomical malformation itself, possible lack of normal neural development (due to lack of closure of the neural tube – see Discussion), CSF leakage triggering electrolyte abnormalities or favoring ascending infectious meningomyelitis (for open MCs/MMCs) and possible tethered cord syndrome (TCS).

Tethered cord syndrome: etiology and clinical relevance in LS MCs/MMCs

In early embryogenesis, the spinal cord occupies the entire length of the vertebral canal and the origin of each pair of spinal

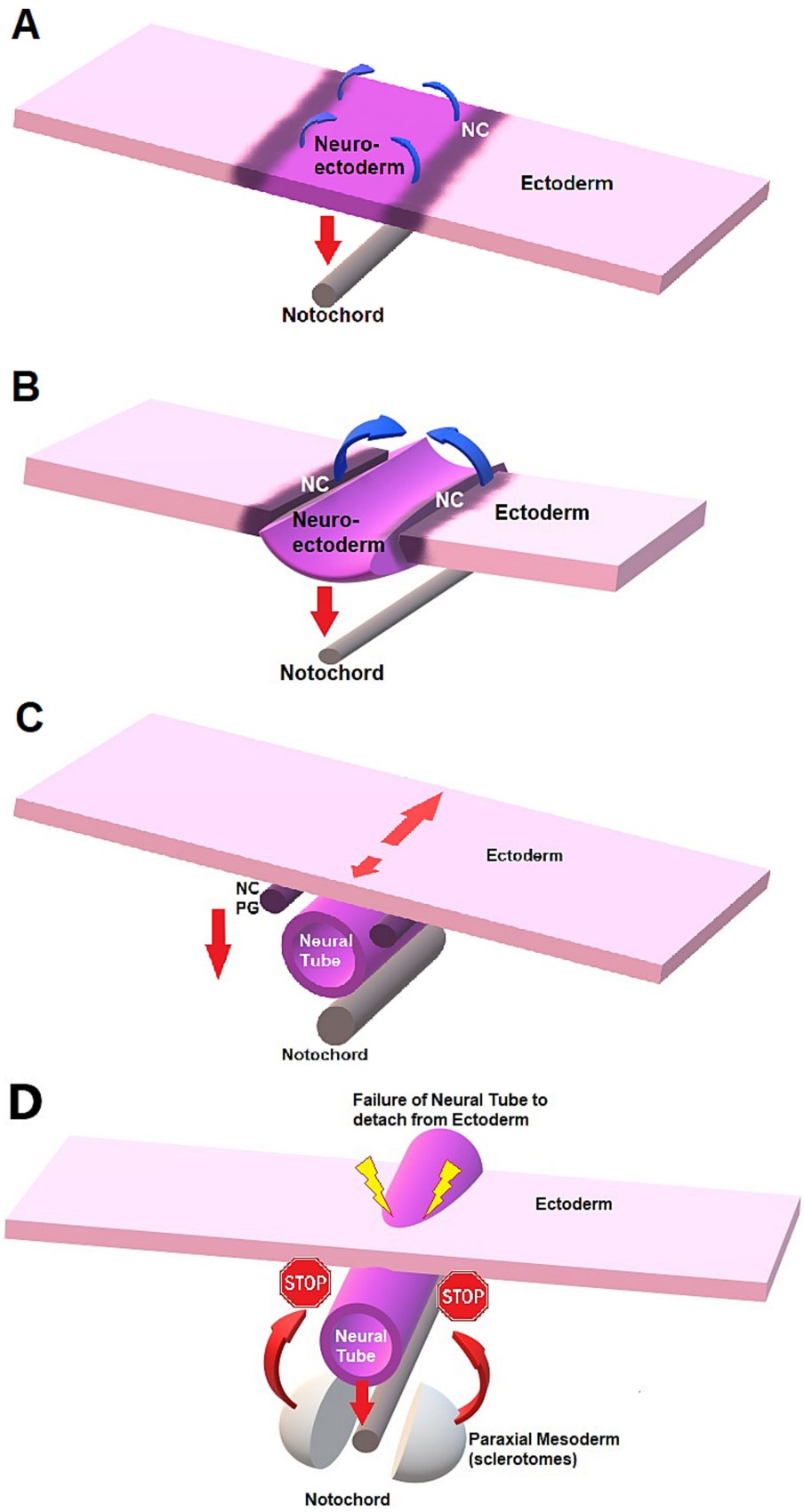

FIGURE 4
Schematic representation of neurulation and failure to detach in NTDs. **(A)** Neural plate stage. The notochord exerts an influence on the neural plate (neuro-ectoderm) for formation of the neural tube through signaling molecules such as sonic hedgehog (*red down arrow*). The ventral aspect of the neural plate bends and is attracted to the notochord ventrally, creating a midline sulcus while the neural crests (NC) cells elevate (*curved blue arrow*).

(Continued)

FIGURE 4 (Continued)
(B) Neural groove. The lateral margins of the neural plate elevate and create the neural folds located at the junction neural plate-ectoderm longitudinally. The folds then get closer to midline until they fuse dorsally, creating a tubular structure: the neural tube. **(C)** As the neural tube detaches from the dorsal, superficial ectoderm to migrate ventrally towards the notochord, the NC cells detach and migrate ventro-laterally on each side. The NC cells will form the dorsal spinal cord and spinal ganglia, after forming the primary ganglia (PG). The number of sites of initial fusion varies per species, and closure proceeds in a zipper-like fashion either unidirectionally or bidirectionally along the long axis, also depending on the species. Neurulation can hence continue cephalad (*short red arrow*) and caudad (*long red arrow*) while the neural tube "migrates" ventrally under the influence of the notochord (*vertical down arrow*). **(D)** NTD and failure of the neural tube to detach from ectoderm, preventing mesodermal structures to fuse on dorsal midline.

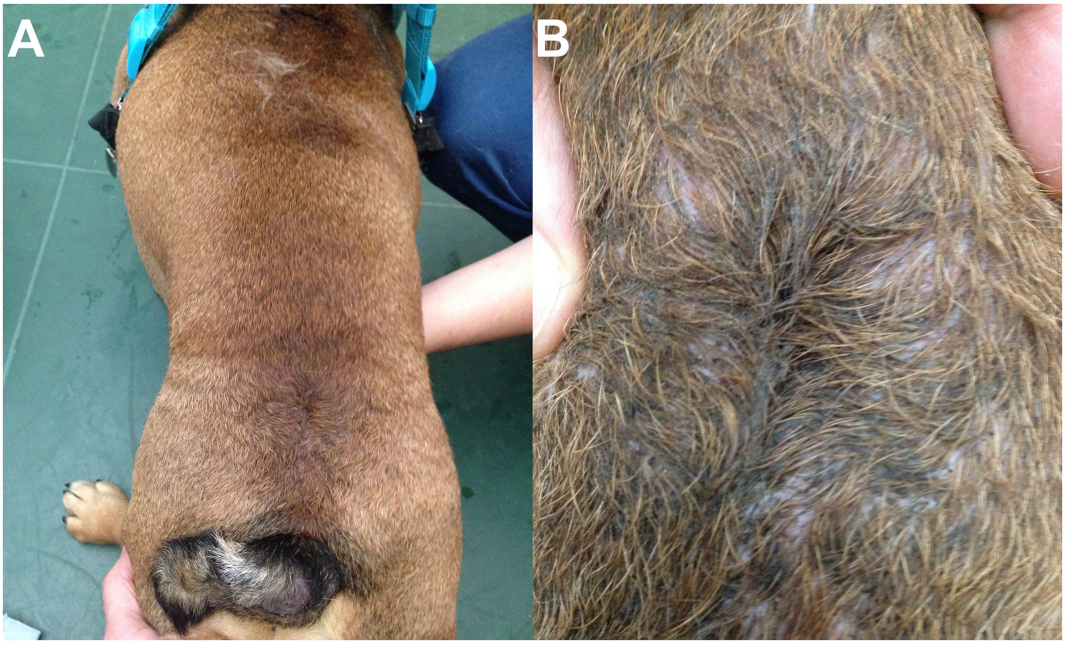

FIGURE 5
1.5-year-old male neutered English Bulldog mix with open lumbosacral (LS) meningocele (MC). **(A)** A swirl of hair and dimple are visible in the dorsal LS area. **(B)** Close-up view showing the CSF leakage and moist aspect of the skin, at the distal, open end of the MC.

nerve roots (dorsal and ventral) along the spinal cord aligns with their exit point of the vertebral column at each respective intervertebral foramen. During embryologic life and post-natal growth until adult size is reached, the spinal cord growth slows down while the rest of the body (e.g., vertebral column) continues to extend longitudinally. This differential in growth in the longitudinal axis between the nervous tissue and the rest of the vertebral column is responsible for a relative ascending movement of the conus medullaris cranially within the vertebral column during growth. Consequently, in an adult mid-size dog, the end of the spinal cord/conus medullaris reaches approximately the sixth or seventh lumbar vertebra, with further cranial locations reported in large dogs (25–27). Hence, the most caudally located spinal nerve roots (lumbar, sacral and caudal) elongate to reach their now relatively more caudal exit point of the vertebral column, creating the *cauda equina*. This ascending movement is allowed by elongation and the elastic properties of the *filum terminale*, a ligamentous structure formed during secondary neurulation and connecting the conus medullaris to the distal sacrum or first caudal vertebra. This differential in longitudinal growth between spinal cord and vertebral column, along with the anchor point of the rostral end of the neuraxis (the brain encompassed in the skull), explain the relative tension/traction put on the conus medullaris if an added anchor point is also present caudally. In NTDs in general and LS MCs/MMCs specifically, the failure of detachment of the neural tube from the ectoderm acts as that anchor point. This progressive and repetitive or sustained traction is responsible for decreased blood flow and decreased oxidative metabolism in the conus medullaris, and potentially more cranial structures (rest of the spinal cord), with syringomyelia as possible consequence (Figure 6).

Tethered cord syndrome (TCS) refers to a constellation of neurological deficits, dermatological, gastrointestinal, urological, and occasionally orthopedic signs, due to abnormal traction on the conus medullaris and spinal cord. This occurs secondary to mechanical tethering from abnormal anatomy (e.g., cases of malformation such as LS MCs/MMCs in bulldogs) or from abnormally inelastic tissue within the filum terminale (28–30). Occult tethered cord syndrome (OTCS) refers to TCS with tethering of the spinal cord by the filum terminale, in the absence of diagnostic imaging abnormalities on static images (including conus medullaris in normal position), and diagnosis may require dynamic views (i.e., comparative images obtained with LS area in flexed, neutral, and extended position) (29, 30). In

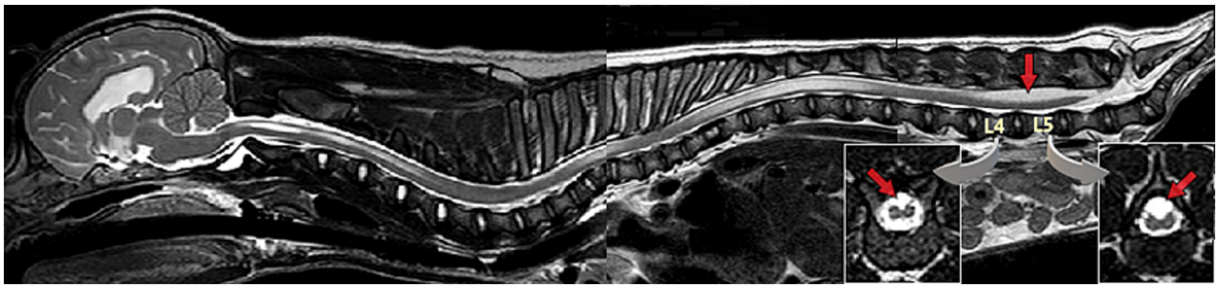

FIGURE 6
Sagittal T2W magnetic resonance (MR) image whole spine & brain (mobiview) of a 3-month-old male French Bulldog/pug mix with spina bifida (SB) at L7 and lipomyelomeningocele with resulting tethered cord syndrome (TCS). Note the area of abnormal unfused/split cord over the vertebral bodies of L4-L5, more conspicuous on transverse images (*inserts*) (see Diagnosis/Diagnostic Imaging for further discussion), the syringomyelia in the thoracolumbar and cervical spinal cord, and the caudal cerebellar indentation.

diagnostic imaging, TCS has been described as a conus medullaris with abnormally caudal position on static images or lacking craniocaudal mobility within the vertebral canal on dynamic views, or as a dorsal displacement of the spinal cord being visualized within or outside the vertebral canal, and can be encountered in other areas of the spinal cord (e.g., thoracic) (28, 29, 31). Cases of TCS are not always associated with the most severe amount of mechanical traction/restriction, but the authors have diagnosed several cases of NTDs with TCS having developed syringohydromyelia in thoracolumbar or even cervical spinal cord on MRI (see Figure 6; see Diagnosis/Diagnostic Imaging/MRI).

Diagnosis

Clinical

Population - signalment

Prior descriptive publications on LS MCs/MMCs report an over-representation of screw tail breeds and Bulldogs specifically (11, 21, 32), as found in the 14 literature cases treated surgically with 12/14 dogs being Bulldogs (8 French Bulldogs and 4 English). The remaining 2 dogs in the literature were a German shepherd dog and a Yorkshire terrier (13–19). All dogs in our cohort were Bulldogs, 6/9 English Bulldogs (including 1 English Bulldog x Pitbull mix) and 3/9 French Bulldogs (including 1 French Bulldog x Pug mix).

Clinical signs are present from birth, specifically the physical abnormality(ies) related to the NTD (see Physical examination below); neurological deficits are also present from birth, although they may worsen with time in cases of TCS and the paresis and incontinence may not be confirmed before several weeks old, when pups in the litter start exploring otherwise. In our cohort, the age of dogs at time of surgery was between 3 and 6 months old with an average of 4.2 months old. The literature cases were between 7.5 weeks and 2 years old, for an average of 5.2 months old, in the 11/14 where age was available (only 1/11 dogs was older than 5 months old – the one who was 2 years old; for a corrected average age at surgery of 3.3 months in 10 dogs if that dog was excluded).

Sex repartition might show a slight male predilection, with 6 males and 3 females in our cohort and 7 males for 4 females in the literature (3/14 sex unspecified). Previous non-interventional studies reported, respectively, 2/3 males (one unspecified) (10) and 18 males for 14 females (11).

Physical examination

On visual examination, patients with LS MCs/MMCs are often juvenile and present an abnormal LS area with various levels of severity. The most common findings are cutaneous signs with a change in hair implantation ("swirl," also reported as streaming of hair) (11) and a depression in the muscles and soft tissues ("dimple"), both on lumbosacral dorsal midline (Figure 7). This was noted in 9/9 authors' cases and reported in 10/14 cases in the literature (in 4 cases the information was missing). For cases of open MC/MMC, the leakage might not be obvious while covered in hair as a crust may form (15); in some cases the area is visibly moist (see Figure 5). This was reported in 1/14 cases in the literature (15) and none in our surgical group. The most severe cases may show a "placode" of neuro-ectodermal tissue as reported in humans, although this is rare in the authors' experience (see Figure 3). In our experience, cutaneous signs suggestive of NTDs are also commonly encountered in the cervical and cranial thoracic areas.

Concurrent malformations

As a rule of thumb with congenital malformations, the earlier in embryology the malformation occurred, the more likely it is to have triggered further anomalies in the embryological development (33, 34). Since NTDs occur early on (neurulation), concurrent malformations – not limited to the nervous system – occur as expected and may be extensive, such as caudal regression syndrome, and/ or include a lethal character (35). Cryptorchidism was present in 2/6 males in our population and 1/7 in the literature (15). Concurrent severe orthopedic issues (joint deformities, severe hip dysplasia) were present in 3/7 cases where that information was available (diagnostic imaging of the pelvic limbs performed or physical examination documenting complete orthopedic examination; in 2/9 cases that information could not be retrieved). Although not included in the surgically treated cohort (owners' decision), the authors have also encountered dogs with external and/or radiographic signs of multiple concurrent NTDs and other malformations, such as severe limbs deformities (see Figures 3, 8), as reported in the literature (10, 11, 36, 37).

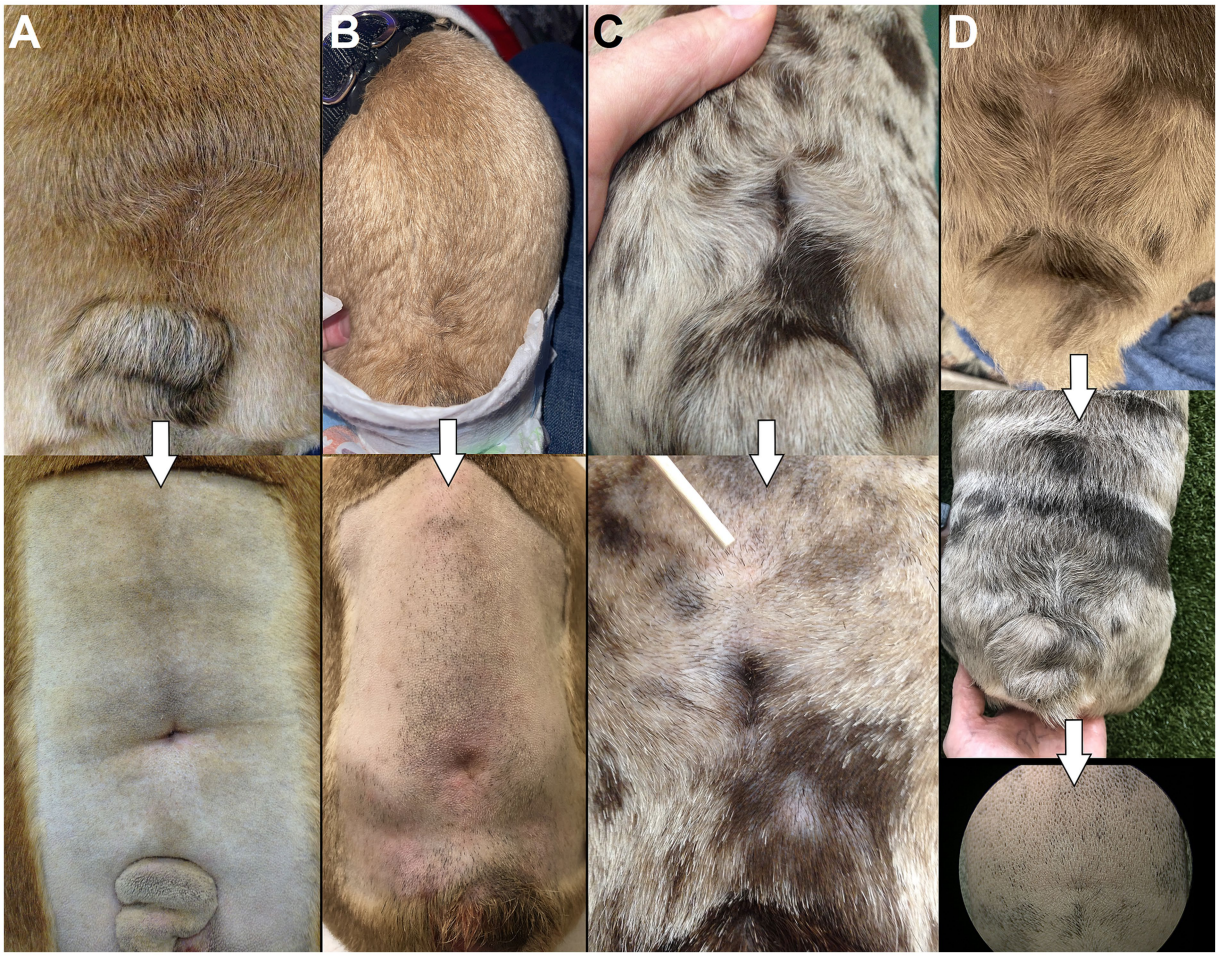

FIGURE 7
"Swirls and dimples". **(A–D)** Examples of the change in hair implantation ("swirl") and depression in the skin/muscles ("dimple") at the site of LS MC/MMC in 4 different bulldog puppies included in our surgical cohort. Top images show dorsal view, bottom images show magnification after being clipped for surgery.

Neurological examination

Neurological deficits reflect the anatomical malformation +/− TCS and most often affect the sacrocaudal nerves with all 9/9 of our patients localizing to an L4-Cd myeloradiculoneuropathy (4/9 showed no deficits of the femoral nerve for a more specific L7-Cd neurolocalization, 2 of which also showed normal withdrawal reflex [decreased in the other 7/9] with normal gastrocnemius reflex in 1 of the 2). Urinary and/or fecal incontinence were reported in all cases (9/9 of our cases and 14/14 in the literature), with some dogs having never voluntarily voided or postured to eliminate to the owner's knowledge. Involvement of the sacral nerves is the rule with abnormal perineal reflex (decreased in 3/9 and absent in 6/9) and anal tone (decreased 3/9, absent 6/9) in all our cases. This was not always retrievable in literature cases but only 1/14 reported intact perineal reflex and anal tone – it is noticeable that this dog was the most caudally affected with the MMC affecting the sacrum and first caudal vertebrae (15). Hypoalgesia or analgesia of the perineal area and/or tail base was also present in 7/9 of our cases (normal sensation in the other 2) and in the 4/14 literature cases (13, 15–17) where information on perineal sensation was reported (no information available in 10/14 literature cases) (14, 18, 19). In the authors' experience, hypoalgesia of the caudal aspect of the pelvic limbs is frequent, although this was not systematically assessed in our first cases. Postural reactions deficits (e.g., proprioceptive placing, hopping) in the pelvic limbs were ubiquitous in our cohort (9/9), although sometimes only mild. Pelvic limbs gait deficits (paresis, ataxia) are frequent in our experience, present in 8/9 cases, albeit only minimal to mild in 5/8 and moderate to severe in 3/8 (one of the 9 dogs presented delayed proprioceptive placing in both pelvic limbs as sole deficits, with no obvious ataxia nor paresis seen on examination). Literature cases account for 10/14 dogs with PLs deficits before surgery varying from minimal to non-ambulatory paraparesis, although specific neurolocalization was not reported consistently (13–19). Another common gait anomaly encountered with NTDs is bunny hoping, present in most patients (7/9 puppies in our cohort) with varying severity and types of gaits affected (walk vs. trot vs. gallop), although infrequently reported on in the literature (13–19). As expected with congenital malformations, pain is not a common feature and although the authors have encountered some dogs showing mild discomfort on LS palpation, this was not a predominant feature in any of the dogs we treated surgically. It was reported in only one dog in the literature (18). Perhaps more surprisingly, signs of neuropathic pain (e.g., dysesthesia,

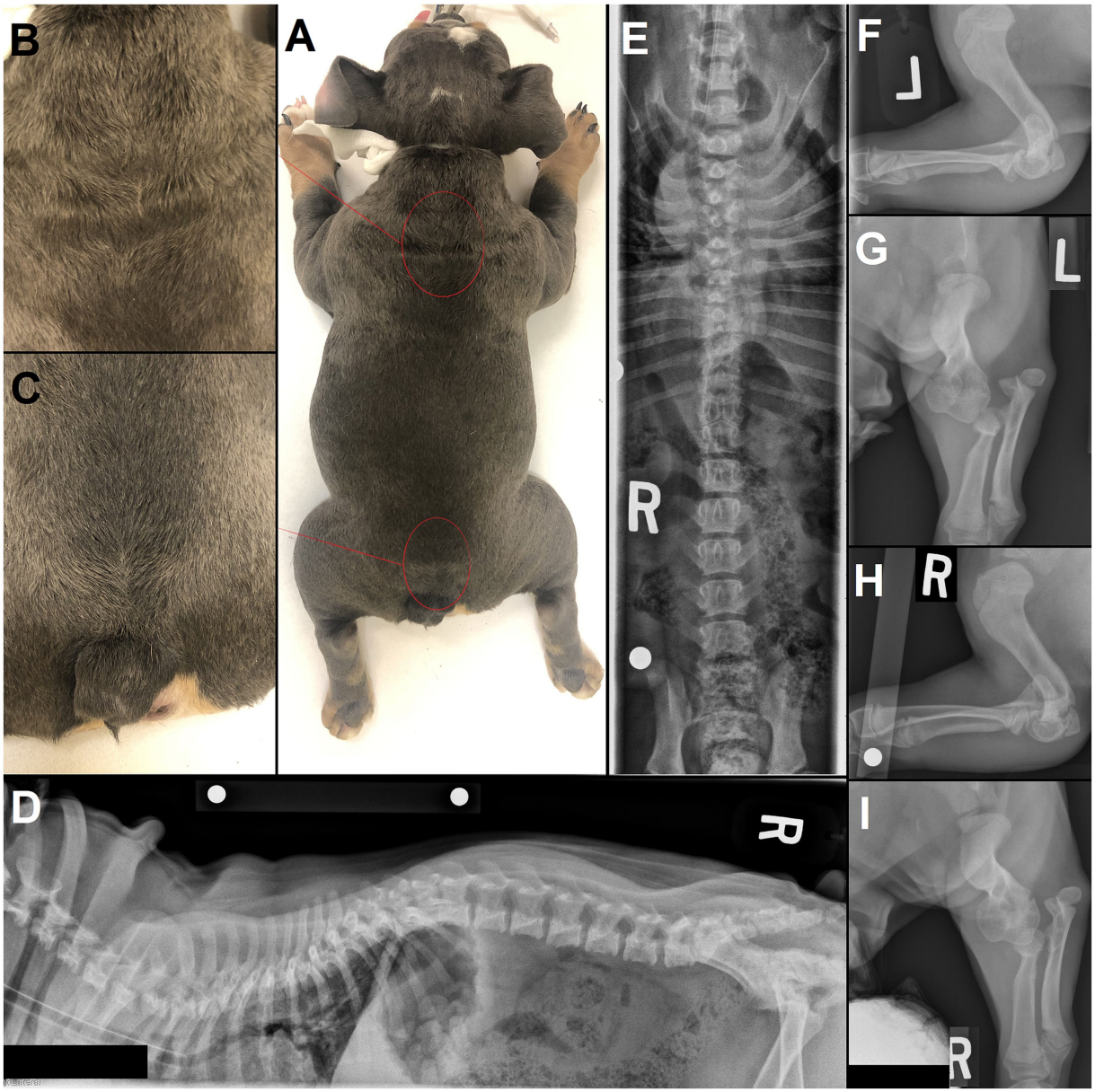

FIGURE 8
4 month-old male English Bulldog with several sites of neural tube defects (NTDs) and concurrent malformations. **(A)** Several areas on dorsal midline show a swirl of hair, most noticeable in the **(B)** upper thoracic and **(C)** lumbosacral area. **(D)** Lateral and **(E)** ventrodorsal radiographs of the vertebral column show an abnormally large spinous process at T1, a bifid, partially fused spinous process at T7 and lack of spinous process at L7 and S1, along with further vertebral malformations (e.g., hemivertebrae and wedge-shaped vertebrae in the thoracic vertebral column, butterfly vertebra with missing right rib at T13). **(F,H)** Lateral and **(G,I)** dorsal radiographs of the left and right elbows show marked vertebral malformations of the thoracic limbs, with severe bilateral elbow dysplasia and luxation.

paresthesia) are not frequently identified in these patients, and were not reported in our group nor in any of the 14 literature surgical cases.

In our experience, clinical signs of TCS with progressively worsening neurological deficits in an ascending pattern was frequent (7/9 puppies), albeit mild and seemingly finite (i.e., last recheck neurological examination was static) in most (5/7). For 2/7 cases with TCS, the last neurological examination before surgery reported minimal/mild worsening, so it could not be ascertained that clinical signs of TCS had stopped worsening. Most of the dogs included in our surgical cohort were monitored for a few weeks prior to surgical intervention, allowing for repeated neurological examination. As reported by previous authors (38), it is our impression that clinical TCS with worsening is frequent in this population at a young age (i.e., between 4 weeks and 16 weeks old), although this was not commonly examined/reported on in the literature. Three single surgical cases reported progression of neurological signs or late onset of signs consistent with worsening TCS (13, 17, 18), while no details specifying progression or lack thereof were found for the other 11 cases (14–16, 19). A common manifestation of progressive TCS would be dogs presenting with initially only sacrocaudal deficits (absent perineal reflex & anal tone, incontinence) showing sciatic nerve deficits at first recheck (e.g., decreased withdrawal in the pelvic limbs at the hock),

and potentially femoral nerve deficits (e.g., decreased patellar reflex, weakness at the stifle) later.

Diagnostic imaging

Radiographs

The osseous part of the malformation (spina bifida) and other concurrent vertebral malformations can usually be seen on routine radiographs, although proper placement is paramount to diagnostic quality. Dorsoventral/ventrodorsal view of the lumbosacral vertebral column is diagnostic for spina bifida if a bifid spinous process can be identified, although technical factors can complicate interpretation (e.g., proper exposition, super-imposition of stools in colon in cases with megacolon) (see Figures 8, 9). It can be difficult to commit to a radiographic diagnosis if the only finding is the absence of a clear spinous process on VD/DV views, without identification of an abnormal, bifid one. Lateral radiographs may also occasionally help identify the skin dimple (see Figure 9). As reported in Bulldogs previously, concurrent vertebral malformations at other sites (e.g., wedge-shaped vertebrae, hemivertebrae, butterfly vertebrae, other sites of SB) are frequent in the authors experience and were found in 9/9 dogs (see Figure 9).

Computed tomography

Location of the main spina bifida site

CT is well-suited for the investigation of NTDs as it allows for a fast examination, doable under sedation-only in a juvenile brachycephalic population, with imaging of the whole body to help in the diagnosis of further malformations. CT is ideal for osseous structures (e.g., spina bifida) and allows 3D reconstruction helpful in visualization of the path of the MC/MMC for surgical planning, with better spatial resolution than MRI (Figure 10). The most common main site of spina bifida for LS MCs/MMCs in our cohort was the last lumbar vertebra in 6/9 cases (5/9 were L7 and 1/9 was L8 due to further malformation) (see Figure 9). Other sites were L6 (1/9), and the sacrum (2/9, 1 affecting only S1, 1 affecting the entire sacrum). Three cases presented spina bifida at 2 or 3 adjacent sites (one at L6-L7, one at L8-S1, one over the entire sacrum) and in 4/9 the first spinous process and lamina directly cranial to the MC/MMC were abnormally shaped (caudal aspect of the lamina oriented dorsally and midline point of the lamina/base of the spinous process appearing shifted cranially due to the MC/MMC) (see Figure 10). Literature cases report specifically the site of spina bifida in 8/14 cases with the sacrum being the most common site (5/8), followed by the last lumbar vertebra L7 (3/8,

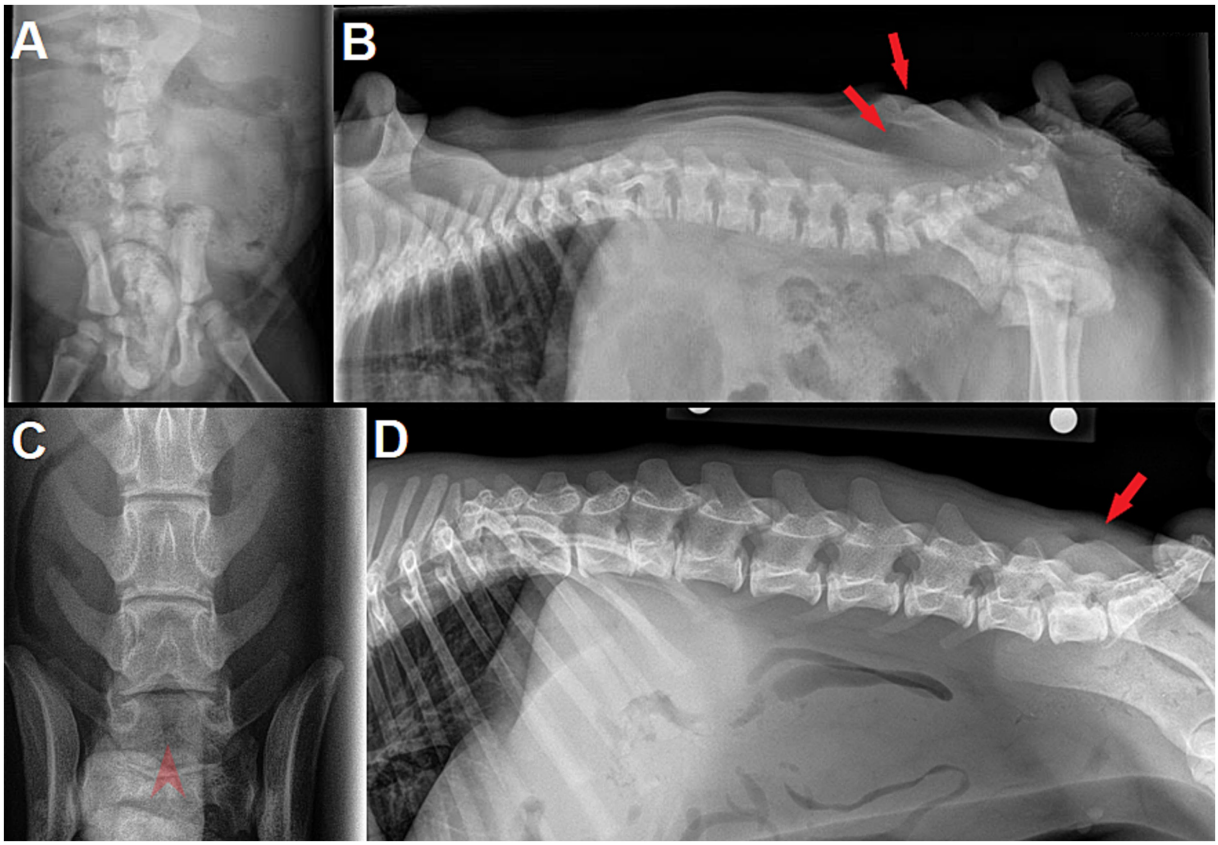

FIGURE 9
(A) Dorsoventral (DV) and **(B)** lateral radiographs of a 9 week-old male English Bulldog with myelomeningocele (MMC) and spina bifida (SB) at L6 (dog D in Figure 7). Note the abnormally small silhouette of the spinous process at L6 on lateral view, along with the skin fold and a mild radiolucent line at the level of the dimple (*red arrows*). Super-imposition of fecal material in the colon prevents proper visualization on DV view. **(C)** Dorsoventral and **(D)** lateral radiographs of a 5 month-old male French Bulldog with MMC and SB at L8 and S1. Note the clear visualization of 2 unfused, hemi-spinous processes at L8 on DV view (*red arrowhead*), while the skin dimple is not as obvious on lateral view (*red arrow*).

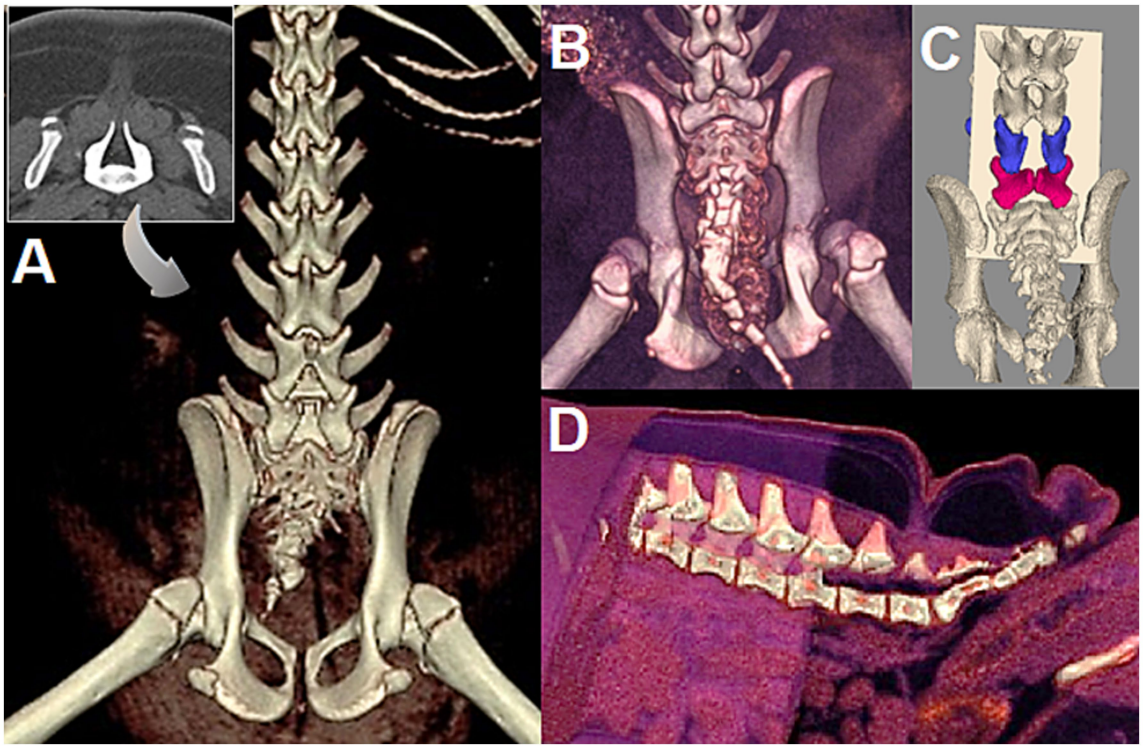

FIGURE 10
(A) 5-month-old female English bulldog (Dog A in Figure 7), CT 3D reconstruction and 2D transverse view *(insert)* at site of spina bifida (SB) at L7. Note the abnormal spinous process/lamina at L6 and SB in the caudal vertebrae. **(B)** 5-month-old female English bulldog (Dog C in Figure 7), CT 3D reconstruction with SB at S1. Note the SB of caudal vertebrae and severe hip dysplasia/incongruency. **(C)** 3.5 month old male English Bulldog (Dog D in Figure 7, radiographs in Figures 9A,B), CT 3D reconstruction. Note the SB at L6 *(blue)*, L7 *(magenta)*, and the slanted forward lamina of L5 *(courtesy of Dr. Sarah Pownder, DACVR)*. **(D)** Same dog as in A, 3D reconstruction of CT allows to cut out anatomical areas to help visualize the path of the MC/MMC for surgical planning.

with 1/3 being L7 + S1) (13, 15–19). In one cohort of 6 dogs in the literature, spina bifida was reported consistently at an intervertebral space/two consecutive vertebrae, either L6-L7 (3/6) or L7-S1 (3/6) (14).

Concurrent, separated sites of spina bifida (caudal vertebrae)

In all cases of the authors experience in Bulldogs (9/9), and in all literature surgical cases and descriptive reports where images of the caudal vertebrae were available for review (16, 18, 21, 32), it is noticeable that SB of the caudal vertebrae was present too, whether documented or not (see Figure 10). This is likely the consequence of primary neurulation failure at the caudal neuropore (site of the MC/MMC), hence preventing a normal secondary neurulation, although the mechanism may be specific to screw-tail breeds considering literature report (15) and personal experience of the authors with normal-tailed animals with sacral MCs/MMCs. Anecdotally, the authors have encountered mild/asymptomatic forms at T1-T2 (see Figure 2), as reported previously in Pugs (39, 40).

Magnetic resonance imaging (MRI)

MRI is the imaging diagnostic modality of choice to visualize the presence or lack thereof of nervous element in the MC/MMC, the position of the spinal cord and the conus medullaris both within the vertebral canal and the dural sac, and any associated spinal cord changes. Since they contain CSF +/− nervous structures, MCs/MMCs appear as T2W and STIR hyperintense, T1W and FLAIR hypointense structures continuous with the normal dural sac, protruding through the vertebral canal and directed towards the skin (Figure 11). MRI allows for best visualization of the nervous tissue within the MC/MMC prior to surgery, although in the authors' experience, the intra-operative findings of nervous tissue components within the MC/MMC do not always match with imaging-based expectations (obtained with a 3 T MRI). In our cohort 1 dog presented a MC, 8 dogs MMCs (with 1/8 presenting a lipomyelomeningocele [the distal end of the MMC including a lipoma], see Figure 6).

On MRI, tethered cord syndrome was defined as dorsal deviation of the spinal cord and was present in 9/9 cases in our group and in all 9 cases in the literature where that information was available (in 5/14 cases the information regarding dorsal displacement or not of the conus medullaris on imaging was lacking). Since both the anatomical malformations of the MC/MMC and the deviation of the nervous structures are obvious on static images in our experience, no dynamic views were performed in our group; none are reported in the literature either.

The conus medullaris position on MRI was L7 in 7/9 cases (4/7 were in the MMC, 3/7 were ventral to the MMC and still in the vertebral canal) and L6 in 2/9 cases (with the MMC containing nerves but no spinal cord). It was documented in only 4 cases in the literature, all reporting it in the MMC at surgery (sacrum for 3 cases, L7 for 1).

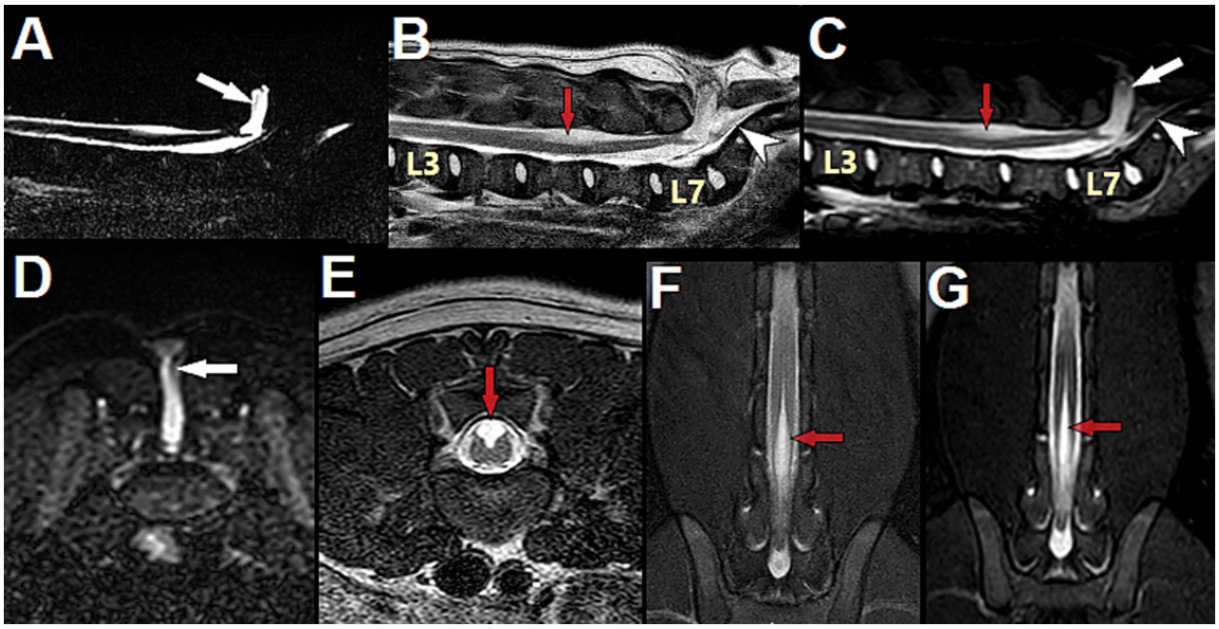

FIGURE 11
3-month-old male English Bulldog with myelomeningocele (MMC) and spina bifida (SB) over the sacrum, magnetic resonance (MR) images. **(A)** Sagittal MYELO, **(B)** Sagittal T2, **(C)** Sagittal STIR, **(D)** Transverse STIR at S1, **(E)** Transverse T2 at L5, **(F)** Dorsal T2 mDIXON and **(G)** Dorsal STIR at the level of the dorsal horns. Note the abnormal structure present in the MMC and tethering the conus medullaris to the distal end of the MMC (thin vertical grey line in **(A,C,D)** *white arrow*). On sagittal images, the conus medullaris and filum terminale internum are present in the normal dural sac, underneath the MMC, while the filum terminale externum is seen caudal to the MMC (hypointense horizontal line in the vertebral canal at the level of the sacrum, *white arrowhead*). Note the central cord hyperintensity suggestive of hydromyelia at L3, and the area of dorsal hyperintensity associated with the unfused dorsal cord at L5 (*red arrow*). Note the open dorsal median sulcus **(E–G)** and the peculiar aspect of the unfused dorsal spinal cord on transverse view **(E)**, showing the failure of midline fusion of the alar plate characteristic of spinal dysraphism (different than syringomyelia).

The filum terminale internum is in our experience most commonly at the base/bottom of the MC/MMC without being in it, while the filum terminal externum is usually caudal to it, in the vertebral canal at the level of the sacrum (see Figure 11).

Further changes in the nervous tissue, signs of TCS and NTD/spinal cord dysraphism, were identified in our cohort such as: *syringohydromyelia* in 5/9 cases (sometimes all the way to the cervical spinal cord), signs of Chiari-like malformation (CLM) regarding the position of the cerebellum and ventriculomegaly/hydrocephalus in, respectively, 2/6 and 5/6 cases where MRI of the brain was performed (see Figure 6), and an open dorsal median sulcus/failure of fusion of the spinal cord on dorsal midline in all MMCs (8/9) (although only minimal/mild in 2 of the 8) (see Figures 11, 12). This last finding correlates with previous reports of structural changes to the dorsal midline of the spinal cord on histology: lack of dorsal median septum, disruption of the ependyma with elongation of the central canal in a slit-like cavitation in the dorsal funiculus (32), and eventually obliteration of the later resulting in an "open" cord covered dorsally solely by a remnant of pia matter (11). This may be difficult to differentiate from *syringomyelia* on MRI as both appear as intramedullary T2W hyperintense, T1W hypointense and FLAIR suppressing areas (although the transverse appearance is sometimes unequivocal – see Figure 11E), and we cannot ascertain that the separate sites of syringomyelia seen in 5/9 of our cases and attributed to TCS (all 5 cases presented worsening of signs) did not represent several sites of failure of proper formation of the neural tube, since NTDs frequently affect multiple areas. If so, the term *hydromyelia* originally defined as dilation of the central canal might be more appropriate and anatomically accurate, since NTDs imply an abnormal/improperly closed central canal (the term *syringohydromyelia* indicates uncertainty regarding communication with the central canal) (see Table 1). Although the term syringomyelia was used in previous reports featuring similar images of unfused/split cord on dorsal midline usually cranial to the MMC (14, 20), it is our impression that these changes are reflective of the nature of NTDs, with a lack of fusion of the 2 dorsal hemi-cords on midline, rather than true syringomyelia (i.e., acquired pathology whereby a syrinx develops in the spinal cord parenchyma without communication with the central canal), which can be seen as a result of TCS.

None of the cases in our cohort showed a true dermoid sinus associated with the MC/MMC. A series of 3 cases report dermoid sinus but no imaging details were available for review (19). Despite the term being used in the literature, there is no detailed advanced imaging or histological report of a true dermoid sinus associated with a LS MC/MMC in dogs to our knowledge thus far, although dermoid sinus have been reported in other areas of the neuraxis, including the head (e.g., nose) (see Discussion).

Treatment options and prognosis

Medical management

Treatment

Medical management for LS MCs/MMCs is limited to palliative care regarding the various pathologies and complications associated:

management of urinary tract infections, possible rectal prolapses, dermatopathies associated with urinary/fecal incontinence, diet adjustments to help manage fecal incontinence (aiming for low-volume of formed feces), management of neurological deficits (e.g., use of cart to help with pelvic limbs mobility) and associated skin excoriations. Pain is not a predominant feature of this disorder in our experience, but anti-inflammatory for local discomfort at the MC/MMC site and gabapentin +/− amantadine for neuropathic pain may be warranted. Antibiotics may be warranted for cases of open MC/MMC (in case of ascending infection).

Outcome

There is a paucity of reports of long-term results of medical management of dogs with LS MC/MMC in the literature, with information being limited to the history until presentation since most dogs were euthanized in early studies (11, 32). Although occasionally described and thought of as static, reports of LS MC/MMC in the literature also describe possible progressive worsening of neurological deficits in the absence of surgical correction (21, 38). This has been our experience, with personal communication with volunteers in Bulldog rescue organizations reporting progressive worsening of paraparesis and para-ataxia over several months/year in some dogs not corrected surgically, although a plateau of neurological deficits where things remain static is most often reached. The continence may also worsen, with some cases suffering initially from only partial/occasional urinary or fecal incontinence progressing to complete urinary and fecal incontinence over time. This also fits with the finding of 7/9 dogs presenting with worsening clinical signs and clinical TCS in our study. While dogs with only minimal, non-progressive neurological deficits may not warrant specific treatment, animals more affected do.

Surgical management

In general, the goals of surgery for MC/MMC should be to:

- Reestablish normal position of the nervous structures in the vertebral canal.
- Untether the nervous structures and meninges:
  - Remove adhesions of the dural sac with periosteum of adjacent laminae.
  - Remove adhesions of the nervous structures (within the dural sac) with arachnoid/dura if present.
- Close the dural defect (for open MC/MMC).

Procedure

The surgical procedure performed by the author is described below and imaged step-by-step in Figure 13.

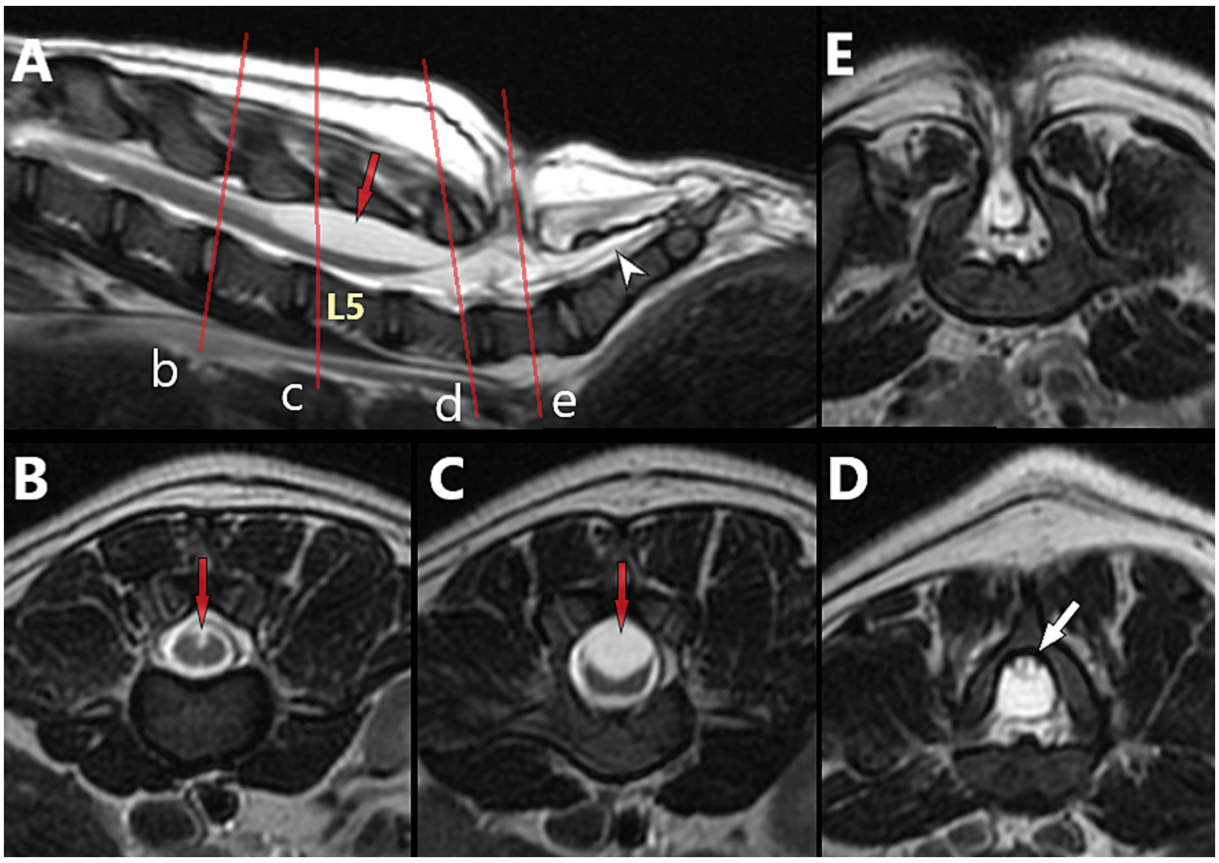

FIGURE 12
5-month-old male French Bulldog with myelomeningocele (MMC) and spina bifida (SB) at L7 (dog B in Figure 7), T2-weighted magnetic resonance (MR) images. **(A)** Sagittal view with indications of the level of the transverse images (*red lines*) at L4 **(B)**, L5 **(C)**, caudal L6 **(D)** and into the MMC at L7 **(E)**. Note the characteristics of neural tube defect (NTD)/spinal cord dysraphism with the unfused dorsal median sulcus over L5 (*red arrow*), and the TCS with dorsally deviated dural sac and conus medullaris at L6 (tucked against the lamina, *white arrow*), suggestive of adhesions between the dorsal aspect of the dural sac and the ventral aspect of the lamina of L6 (see Figures 14C–E for intra-operative images).

The patient is clipped and aseptically prepared for surgery on dorsal midline from ~L3 to the tailbase, over a width slightly larger than the wings of the ilium. If fecal leakage is significant, a purse-string suture can be used for the time of the surgery. Positioning is in sternal recumbency, with pelvic limbs forward and support (e.g., surgical towels, bean bag) under the pelvis to maintain stability. Cutaneous incision starts with a round or elliptical incision centered on the swirl of hair and is extended on sagittal midline cranially and caudally (Figures 13A,B). The subcutaneous and fat tissues are dissected until a cylindrical structure underlying the area of deepest cutaneous depression can be identified. Dissection is continued along the stalk of abnormal fibroadipose tissue as it "dives" between the lumbar muscles and reaches the lumbodorsal fascia, using a combination of blunt and sharp dissection (cotton tip applicators can be used to "brush off" the softest fibrous tissues from the stalk, Castroviejo, iris and corneal scissors for sharp dissection of the toughest tissues) (Figure 13C). The change in orientation of the lumbodorsal fascia fibers mark the dorsal midline defect (*black arrow* in Figure 13C) through which the MC/MMC passes and can be used as landmark to palpate the two unfused spinous processes at the site of spina bifida. The lumbodorsal fascia is then incised around the first normal spinous process just cranial to the defect (e.g., L6 if spina bifida at L7) and the incision is continued caudally around the MC/MMC on each side of the SB site (Figure 13D). The multifidus lumborum (cranially) and sacrocaudalis dorsalis medialis (caudally) muscles are elevated from the first normal spinous process, the two unfused hemi-spinous processes at the site of SB (*black arrowheads* in Figure 13D) and the median sacral crest, and are maintained with Weitlaner and/or Gelpi retractors. In our experience, further attachment points of the MC/MMC to adjacent structures are common (e.g., on dorsal midline, to the ventro-caudal aspect of the dorsal lamina of the normal vertebra just cranial/caudal to the MC/MMC; on lateral aspects, to the hemi-spinous processes at the site of SB). If an attachment point to the lamina of the vertebra cranial (or caudal) to the site of SB was seen on images or at surgery, a partial laminectomy of this vertebra should be performed to remove all adhesions and ensure that no tethering persists. A combination of bipolar cautery on very low setting, Castroviejo scissors and number 11 blade is used to remove all adhesions between MC/MMC and surrounding vertebrae and ligamentous structures (note the difference between E and D, also see Figure 14). The unfused hemi-spinous processes may need to be removed to facilitate manipulation of the dural sac (Figure 13E). This can be done with rongeurs or high-speed drill if the cord is protected by a flat and blunt instrument on the medial aspect of the bone. The stalk of abnormal fibrous tissue and skin covering the distal end of the MC/MMC can be dissected from it using Castroviejo, iris or corneal scissors once a cleavage plan is identified (*white arrow* in Figure 13F), working from normal dura at the SB site towards the abnormal tissue. The end of the dural sac is usually covered by this abnormal tissue and extends deeper into it than initially visible (similar to a mushroom cap covering the stem). The dissection continues in a degloving pattern and is finished circumferentially at the most distal end of the MC/MMC with transection of any "dead-end" malformed nervous structure ending in the skin. The part of the normal dural sac not involved in the MC/MMC and caudal to it (*black arrow* in Figure 13F), including the filum terminale, should be visualized and checked for adhesions. Once the dural sac is freed from all attachments around the MC/MMC, 4 stay sutures are placed at the corners of the MC/MMC prior to performing a circumferential durotomy at the base of it (Figure 13G). The dura of the MMC is progressively everted in degloving pattern to ensure that arachnoid adhesions attaching nervous structures (nerve roots) to the abnormal dura are incised progressively. This is continued until the distal end of the MMC, where any "dead-end" structure (*white arrow* in Figure 13G) finishing in or through the dura should be excised, while any structure folding around to go back down into the caudal dural sac (*black arrow* in Figure 13G) may represent a viable sacrocaudal nerve and should be preserved. Once the abnormal dura is removed, the preserved nervous structures are replaced in the underlying normal dural sac (*grey arrow* in Figure 13H) which should be inspected for further adhesions before closure. The dura is closed with a simple continuous pattern (6–0 Vicryl™) (Figure 13I). A fat pad or Gelfoam® layer is placed over the laminectomy defect and remaining closure is routine.

Intra-operative findings

In our cohort, 1/9 was a MC and 8/9 were MMCs, with 1 of the 8 MMCs presenting a large lipomatous mass at the distal end of the dural sac, in and through the MMC, continuous from the intradural compartment to the subcutaneous tissue (through the meninges), prompting a diagnosis of lipomyelomeningocele (transitional/chaotic lipoma if applying human classification) (Figure 14). Numerous leptomeningeal adhesions between the nerve roots of the cauda equina and the lipoma or dura of the MMC had to be dissected for progressive lipoma removal.

One interesting intra-operative finding was the presence of adhesions between the dural sac/MC/MMC and the surrounding structures (ventral lamina of the normal vertebrae adjacent to the MC/MMC [e.g. L6 or S1 if SB at L7], bifid spinous process, ligamentous structures). Although not documented systematically in our first cases, this seems to be the rule more than the exception in Bulldogs and was responsible for tethering of the cord (visualized by ascending movement of the dural sac in the vertebral canal upon removal) (see Figure 14). There were also occasional adhesions within the dural sac/MMC between the nervous structures and meninges, as reported before (14, 17).

Outcome

The dog diagnosed with lipomyelomeningocele died within 72 h of surgery (found acutely dead with notes documenting bright, alert and responsive (BAR) status <10 min before), leaving 8/9 dogs with long-term follow-up in our cohort (>6 months). Autopsy showed changes in the lungs with pulmonary edema and positive oil red O stain for lipid, prompting a presumptive diagnosis of pulmonary embolism of fat-containing tissue (e.g., lipoma, subcutaneous fat, bone marrow) (see Discussion). Out of the literature cases, 1 dog was euthanized at 2 weeks post-operatively with only minimal improvement reported (16) so it is not counted in outcome (timeframe too short to judge of surgical success or not in our experience). One dog died of parvovirus infection after 1 month post-operatively but had fully recovered continence and improved pelvic limbs deficits at day 15 (14), so is counted in outcome. All other literature cases had ≥3 months follow-up.

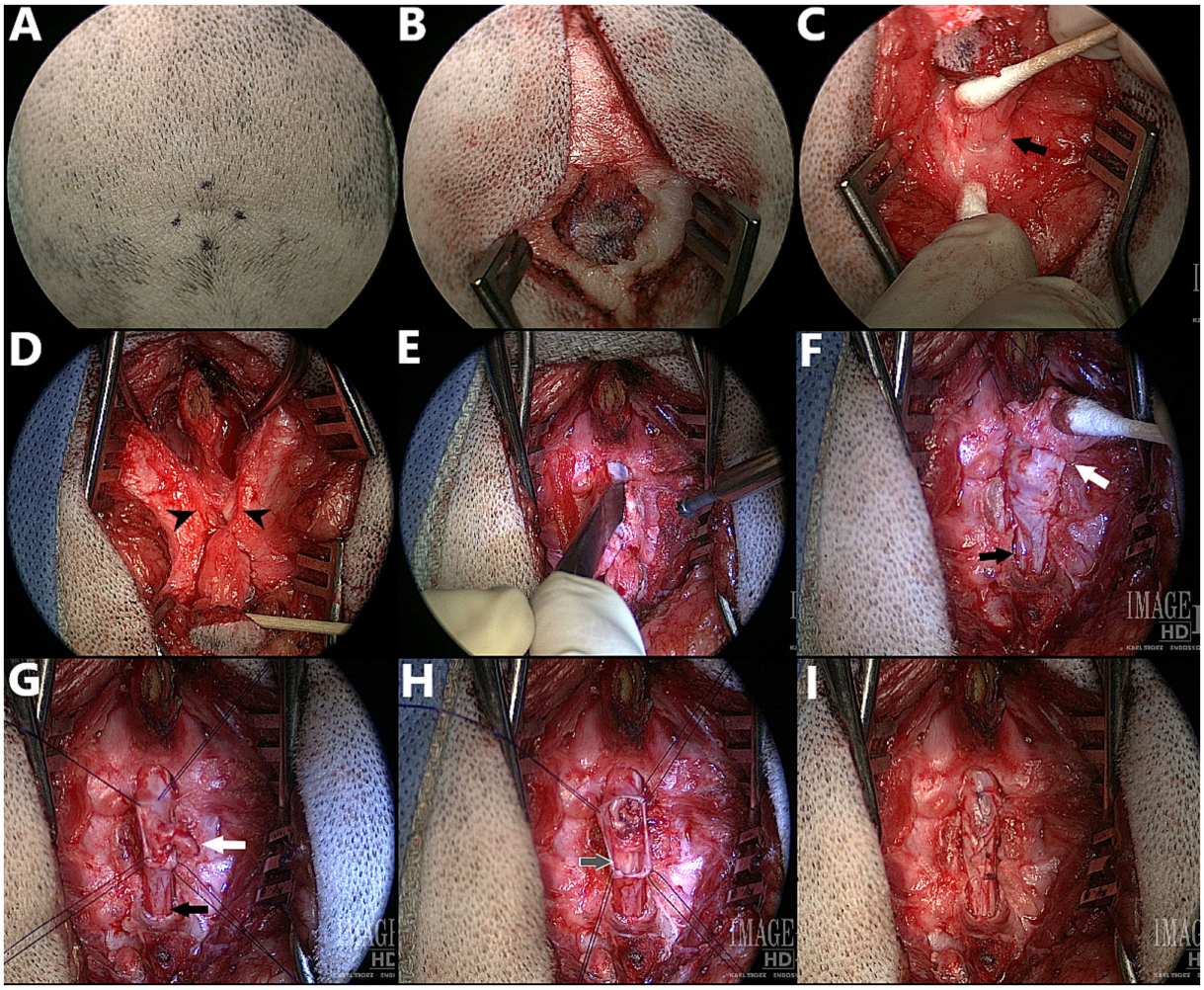

FIGURE 13
Surgical correction of myelomeningocele (MMC) with spina bifida (SB) at L6 in a 3.5 month old male English Bulldog, dorsal view – cranial at the top of the images (Dog D in Figure 7, radiographs in Figures 9A,B, CT in Figure 10C). **(A,B)** Cutaneous incision. **(C)** Dissection around the abnormal fibroadipose tissue (*black arrow* indicates the palpable dorsal midline defect in the lumbar muscles). **(D)** Incision and elevation of the lumbar muscles (*black arrowheads* indicate the two unfused hemi-spinous processes at the site of SB). **(E)** Laminectomy at the site of SB +/– adjacent sites if required for visualization of the MMC. **(F)** Visualization of the MMC, reclined cranially for presentation (*white arrow* indicates the attachment of the fibroadipose tissue onto the distal end of the MMC, *black arrow* indicates normal dural sac caudal to the MMC). **(G)** Durectomy performed (*white arrow* indicates the aberrant nervous structure terminating as a dead-end in the MMC that is resected, *black arrow* indicates the caudal dural sac and continuation as filum terminale externum). **(H)** Durectomy performed (continued) (*grey arrow* indicates the sacrocaudal nerves and filum terminale internum caudal to the MMC; visualized discoloration due to use of Surgicel®). **(I)** Closure of the dura with simple continuous pattern (6–0 Vicryl™) before routine closure.

Regarding neurological deficits, our cohort and literature review document improvement of pelvic limb deficits, respectively, in 6/8 and 8/9 (total 14/17, ~82%) of dogs that had deficits before surgery and follow-up for ≥1 month. It should be noted however that this criterion can be vague and not the most relevant clinically, as motor and sensory deficits are mild to lesser in most cases and rarely the primary concern of the owners, the urinary/fecal incontinence is.

Recovery of urinary and fecal continence can be difficult to accurately report on, as many dogs do not have an all or nothing response, but a partial/incomplete recovery. The criteria we retain in evaluation of our population was to ask the owners if the urinary/fecal incontinence still warranted for the patient to wear diapers at home most of the time or was manageable without (i.e., does the pet still have to practically be managed for incontinence at home). As an example, a dog who only shows occasional feces dropping when running outside with no other sign at home would be considered having recovered. Regarding this criteria, review of our cohort and the literature shows recovery of urinary/fecal continence in, respectively, 4/8 and 6/13 dogs (total 10/21, ~48%). One dog in each group recovered/improved urinary or fecal continence but not the other so the 2 dogs were counted as non-recovery. In the total of 10/21 dogs that recovered, 1 of them died at 1 month of parvovirus infection (14) and 1 of our cohort lost urinary continence again due to a multi-drug resistant *Enterococcus faecium* urinary tract infection (UTI) with penile necrosis at ~6 month post-operatively (penile amputation and scrotal urethrostomy had to be performed), leaving a total of 8/21 (38%) dogs that remain alive and continent at 6 months post-operative.

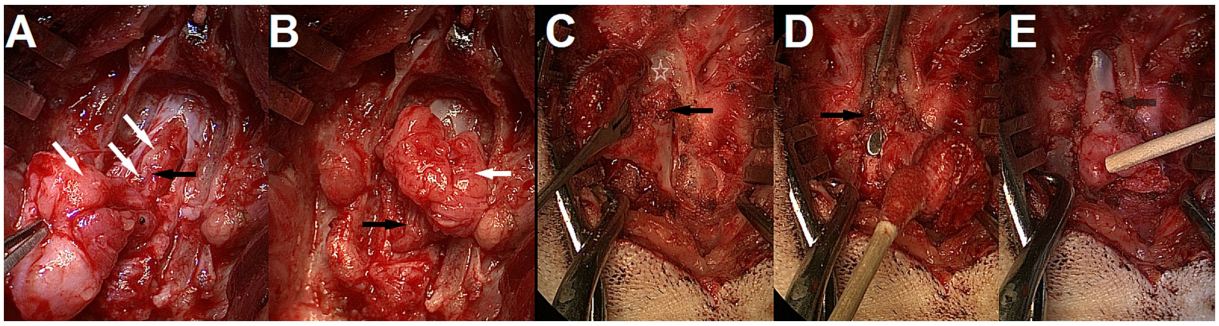

FIGURE 14
Intra-operative findings and adhesions. **(A,B)** Lipomyelomeningocele in a 3-month-old male French bulldog/pug mix with spina bifida (SB) at L7 (MRI in Figure 6). Laminectomy of L6, removal of the bifid spinous processes of L7 and durotomy were performed. Note the cauda equina infiltration by the lipoma (*white arrows*). The sacrocaudal nerves are visible in the lipomatous mass and caudal to it after partial dissection (*black arrows*); they should be preserved. **(C–E)** 5-month-old male French Bulldog with MMC and SB at L7 (dog B in Figure 7, MRI in Figure 12) **(C)** Partial laminectomy of L6 (*white star*), showing thick fibrous adhesions (*black arrow*) between the ventrocaudal aspect of the lamina and the dura/MMC. **(D)** Laminectomy performed at L6 with spatula between the dural sac and adhesions (*black arrow*) for visualization. **(E)** Removal of adhesions complete, the attachment point can be seen (*see-through black arrow*).

Discussion

The exact nature of the fibrous tissue between the distal dural end of the MC/MMC and the skin is unclear to our knowledge. Although dermoid sinuses have been documented histologically in MCs/MMCs of other areas of the vertebral column (41), it is not our experience that dogs with LS MCs/MMCs have a true dermoid sinus associated. The term was mentioned in a previous surgical report, with the authors taking the precaution to report a "dermoid sinus-like lesion" since no lumen was reported (18). All types of dermoid sinus, including suggested type VI, comprise at least in the superficial part a tubular structure with findings of follicular annexes and/or sebum in its lumen at surgery (42, 43). This is not our surgical experience. Although no histological analysis was performed in our cohort, gross macroscopic impression is the one of abnormal fibrous & fatty (fibroadipose) tissue, without any tubular, hollow structure required for the qualification of dermoid sinus. It is possible that the appearance of the sagittal views on CT/MRI images with the skin creating a dimple/well at the attachment point of the MC/MMC (see Figures 10, 12) has led to the erroneous use of that term, along with the appearance of the cylindric fibrotic structure connecting the MC/MMC to the skin (since the surgical act of dissecting along a hollow tube or a plain cylinder is similar). It is also possible that this abnormal tissue constitutes a new subtype of dermoid sinus devoid of lumen and adnexal structures entirely, but this is unreported and contrary to the current definition of dermoid sinus. Published reports with autopsy/histopathology available do not describe a dermoid sinus (32) or lack description of skin changes (11). One series of 3 dogs mention dermoid sinus but no specific information on images or histology could be retrieved, and no mention of any hollow structure containing adnexal structure/sebum is made (19).

Post-operative complications and outcome

Review of our cases and literature reports 8/9 + 14/14 (22/23, ~96%) dogs that underwent surgery and recovered without major neurological worsening, although 1 dog in our cohort died <72 h after surgery. Considering the presumptive diagnosis of fatty pulmonary embolism (see Treatment/Surgical management/Outcome) and the extensive dissection of a lipomatous mass that was required at surgery (see Figures 14A,B), this should be considered a possible complication for surgical repair of lipomyelomenigocele (although the origin of the fatty embolism cannot be ascertained, a bone marrow origin seems less likely in a juvenile patient). Temporary neurological worsening was observed in the immediate post-operative period (<48 h) in 1/9 dogs in our cohort and 2/14 in the literature (14), all back to pre-operative status or improved after 48 h. Regardless of other unrelated death/complications, no long-term neurological worsening has been reported thus far, hence the surgery appears overall safe from a neurological standpoint.

Although there is a lack of medically treated control group to compare, the recovery rates of ~48% for urinary/fecal incontinence and ~ 82% for pelvic limbs deficits appear favorable to recommending surgical management in most cases. It remains unclear if earlier surgery would be associated with a better functional and overall outcome. On one hand, human studies have clearly shown the benefits of earlier intervention in development, leading to the current consensus of intra-utero surgery since the Management of Myelomeningocele Study (MOMS trial) (7); on the other hand, in-utero surgery being unrealistic in veterinary medicine in the near future, one must consider the risks involved with anesthetizing the typical patient, already brachycephalic and juvenile, at an even younger age.

Regarding the recovery of urinary/fecal continence, we have not been able to isolate a possible influence of age at surgery, severity of symptoms, nor imaging changes (such as position of the conus medullaris). Although reported in terms of continence vs. incontinence, the post-operative urinary/fecal functions rarely fall within a purely dichotomic classification of normal vs. abnormal. Several dogs considered to remain urinary/fecal incontinent did show signs suggestive of an improvement of the urinary function, such as easier bladder expressions, bladder found empty on recheck examinations and/or absence of UTIs post-operatively (12, 13, 16). Similarly, the multi-drug resistant *Enterococcus faecium* UTI with penile necrosis

that led to scrotal urethrostomy and loss of regained urinary continence in one of our cases might be a sign of incomplete urinary recovery, since a similar case of penile necrosis was reported in a medically managed 5 month old Cocker Spaniel with MMC and SB at L7 (10).

The significance of the open dorsal median sulcus documented previously (11, 32) and in 8/9 patients on MRI in our cohort (see Figures 6, 11, 12) from a developmental, neuro-anatomical and recovery standpoint is unclear. It is possible that this sign of NTD/spinal dysraphism be accompanied with further anomalies of neuronal migration and neuronal architecture of the spinal cord, which might preclude recovery even after gross anatomical correction (i.e., this aspect of the pathophysiology of LS MCs/MMCs may be more relevant for continence prognosis than the TCS). Although small numbers do not allow to draw conclusion, the 1/9 dog who did not present this finding (the only MC) and 2/9 that were qualified of only minimal/mild changes were all within the 4 dogs of our cohort who had the best recovery (both pelvic limbs function and continence).

Surgical technique

The surgical technique utilized by the authors and the ones described in the literature are sensibly similar with a common dorsal midline approach, dissection around the tract of the MC/MMC followed by durotomy/durectomy, transection +/− removal of abhorrent structures and closure (12–19). Several questions regarding best surgical options persist. The adhesions found between the dural sac and adjacent structures (most commonly ventral lamina of the normal vertebra cranial to the MC/MMC) were responsible for tethering, so we believe they should be identified on images and addressed surgically with partial laminectomy of affected vertebrae. Interestingly, we have not made the same observation in cases of feline LS NTDs that we treated surgically. Intradural adhesions between meninges and nerve roots present in the MMC also require dissection as reported before (14), which can be surgically challenging; this phenomenon was most severe in the case of lipomyelomeningocele in our experience (see Figure 14).

Re-tethering is a documented complication in human LS MMC surgical repair and, although it has not yet been described in veterinary medicine, there is no report of imaging follow-up post-operatively in dogs aside of a partial one at 8 weeks (20). Filum terminale transection has been used successfully to treat TCS in humans after LS MMC surgery and in veterinary patients with occult TCS (28–30). The role of the filum terminale in the pathophysiology of canine LS MMC, hence the surgical attitude to employ, are unclear. Surgical practices in humans focus on transection of the filum terminale internum (FTI) following durotomy, with authors arguing that transection of the filum terminale externum (FTE) is unlikely to have significant effect on TCS due to lack of impact of FTE tension on the position and morphology of FTI (44). By opposition, all reported cases of occult TCS in the veterinary literature and cases treated successfully by the authors all underwent transection of the FTE only (28–30). Filum terminale transection was mentioned in several literature cases of MC/MMC (13, 14, 16), however surgical reports describe the transection of the abnormal cul-de-sac structure present in the MMC rather than true section of the FTI (in our experience not located in the vertical part of the MMC, but in the dural sac present at the base of the MMC or caudal to it) or FTE (usually present caudal to the MMC, in the vertebral canal of the sacrum, and requires laminectomy of the sacrum for visualization) (see Figures 11–13).

Limitations

This review has several limitations. First, only the English literature was reviewed, so reports in other languages may have been ignored. Second, although the authors included every dog treated surgically until the redaction of this manuscript (i.e., no dog who underwent surgery was excluded), the overrepresentation of case reports and very small case series in the literature introduces the risk for several biases: selection bias, lack of consecutive sampling (aside of our cohort), lack of prospective data collection in favor of retrospective description, and the absence of control group. Other limitations include the lack of imaging follow-up (preventing conclusion regarding restoration of normal anatomy and evolution of the dysraphic area of nervous tissue), the lack of histological analysis of the cul-de-sac nervous structure resected at surgery (preventing conclusion regarding the exact nature of this tissue), and the lack of neurophysiological assessment through electrodiagnostics.

Intra-operative neuromonitoring of human patients undergoing tethered cord release after having undergone intra-utero MMC closure (as fetuses) confirmed a motor-sensory discordance with altered somatosensory pathways (45). This is likely related to the pathophysiology of NTDs (affecting specifically the dorsal part of the neural tube) and reflective of further disturbances in the formation of the alar plate, which might present a challenge for recovery of continence. In humans, urodynamic testing can help predict some complications such as upper urinary tract changes related to myelodysplasia (46), while EMG of SB patients has shown changes in the tibialis anterior, extensor digiti brevi, gastrocnemius medialis and external anal sphincter (the latter being significantly more affected in *spina bifida aperta*) (47). Needle EMG of the external anal sphincter and the tail base muscles of a 2-month-old English Bulldog who did not recover continence post-surgery showed absence of any insertion potential or spontaneous activity (13). EMG of the pelvic limbs was normal in this dog and in 1 out of 2 bulldogs treated with mesenchymal stromal cells; it showed mild changes in the other, while both sensory and motor nerve conduction velocities were within normal limits (20). EMG of the external anal sphincter and somatosensory testing might represent prognostic indicators and this should be evaluated in future canine studies.

Summary and conclusion

Lumbosacral MCs and MMCs are congenital malformations affecting preferentially screw-tail breeds (e.g., Bulldogs), often associated with tethered cord syndrome and potential worsening of neurological deficits at a young age. Due to the specific pathophysiology of neural tube defects, these patients present a constellation of neurological deficits (urinary/fecal incontinence, bunny hopping, pelvic limbs deficits) and external signs (e.g., swirl of hair with dimple in the skin +/− lumbar muscle) that is pathognomonic of their condition. Ideal imaging recommendation for surgical planning is, in order, MRI + CT > MRI > CT. Other co-malformations, such as orthopedic issues and cryptorchidism are

common. Surgical correction can be challenging, specifically in cases of lipomyelomeningocele, but appears overall safe with 22/23 dogs surviving the post-operative period and no case of major worsening yet reported. While the results might be biased positively due to the absence of prospective study and to the hegemony of case reports and small case series, post-operative neurological improvement/recovery is possible, although it is less frequent for urinary and fecal continence (<50%) than for pelvic limbs deficits (~80%). Future studies are required to identify prognosis regarding recovery of fecal and urinary continence. It is recommended to address adhesions of the dural sac with adjacent bones (usually ventral aspect of the lamina) and leptomeningeal adhesions within the dural sac during surgery, in an effort to release the tethered nervous structures.

Author contributions

PR: Writing – original draft, Writing – review & editing, Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization. CD: Writing – original draft, Writing – review & editing, Investigation.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The authors declare that no Gen AI was used in the creation of this manuscript.

Publisher's note

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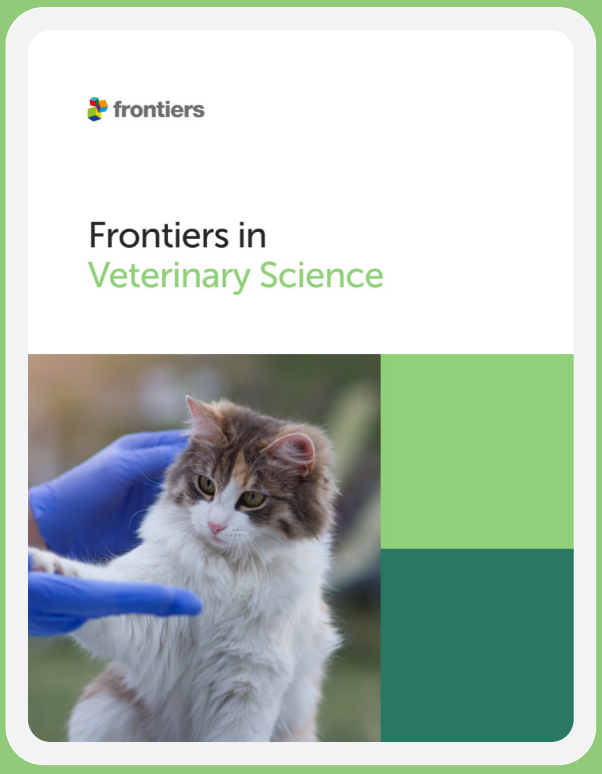

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