DISSERTATION

EQUINE CERVICAL PAIN AND DYSFUNCTION

Submitted by

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ABSTRACT

EQUINE CERVICAL PAIN AND DYSFUNCTION

Cervical pain and dysfunction in horses has become more recognized in recent years. However, a horse may present with a long list of different clinical syndromes and the examination findings can be confusing, resulting in difficulty effectively treating the horse. This frequently leads to frustration by the owner, as well as the veterinarian charged with helping the horse. This body of work aims to enlighten the reader of the dearth of understanding of cervical pain and dysfunction, to highlight how dangerous behavior may be related to cervical pain, and describe the course and development of future research.

There is a paucity of peer-reviewed equine literature available describing cervical pain and dysfunction in the horse. The first chapter is designed to provide a synopsis of the current state of understanding of the disease processes, diagnostic capabilities, and possible treatment strategies available to manage cervical pain and dysfunction in horses.

The second chapter describes a series of horses displaying unwanted behavior that became dangerous to the rider and often times to the horse itself. The included horses all had moderate to severe ganglionitis at multiple vertebral levels. Ganglionitis has been associated with neuropathic pain in other species, and is believed to be causing a state of neuropathic pain in this series of horses. This study highlights the need for deeper understanding of pain behavior in horses.

Chapter 3 describes a prospective evaluation of cervical pain and dysfunction in 12 horses. Recombinant equine interleukin-1β (reIL-1β) has been used as an acute synovitis model within the appendicular skeleton and was utilized in this study to create transient synovitis at the cervical articulation of C5-C6. This study evaluated the clinical, biomechanical and ultrasonographic features in horses with a known source of neck pain. Acute synovitis of the articular process joint (APJ) induced clinical signs of myofascial pain and neck stiffness with variable degrees of forelimb lameness. Ultrasonographic evidence of the presence and severity of APJ effusion could be readily identified and tracked over time. Utilizing this model in the future could further add to our understanding of the clinical presentations in horses experiencing cervical pain and dysfunction.

Through this collection of work, we have developed collaborations to investigate many unanswered questions that have been raised. We will look to define pathways related to neuropathic pain mechanisms in order to ultimately improve the quality of life, not only for our equine patients, but potentially of other veterinary species and even the human population experiencing chronic pain.

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EQUINE CERVICAL PAIN AND DYSFUNCTION: PATHOLOGY, DIAGNOSIS AND TREATMENT[1]

1. Introduction

It is becoming increasingly recognized that many horses presented to equine practitioners for poor performance have underlying cervical axial skeletal lesions that result in pain syndromes and an inability to meet athletic demands. However, understanding exactly which structures within the cervical region are affected remains difficult and a potential source of frustration. Unfortunately, diagnostic imaging modalities often fail to help to fully elucidate the underlying disease process, which is similar to what is seen in human patients (1). The prevalence of neck pain in humans ranges from 30% to 50% (2) and appears mostly associated with abnormal joint motion and disc degeneration (3-5). However, human physicians also struggle to identify the source of neck pain even after employing advanced imaging modalities or other diagnostic techniques and obtaining verbal feedback from their patients. This underscores the challenges we face in equine practice to understand and diagnose this frustrating and potentially debilitating condition in horses. Due to the paucity of peer-reviewed equine literature on this topic, the information discussed here is a hybrid of a literature review, which includes human and other animal species as needed to delineate specific concepts, combined with the authors' clinical and research experience. The goal is to provide a synopsis of the current knowledge of common disease processes, diagnostic approaches, and treatment strategies used for managing cervical

[1] This chapter has been published: Story MR, Haussler KK, Nout-Lomas YS, Aboellail TA, Kawcak CE, Barrett MF, Frisbie DD, McIlwraith CW. Equine Cervical Pain and Dysfunction: Pathology, Diagnosis and Treatment. *Animals* (Basel). 2021 Feb 6;11(2):422. doi: 10.3390/ani11020422. PMID: 33562089; PMCID: PMC7915466.

pain and dysfunction in horses. It is meant to highlight the many topics and considerations when dealing with a horse presenting for concerns related to the neck. As information is getting added to the literature at a rapid rate, it is important for veterinarians presented with these types of cases, to stay abreast of new material. It is the authors' opinion that as more and more practitioners and riders begin to recognize the complexity of these cases, we can work together to ultimately improve the clinical outcome of these challenging cases.

2. Pain Mechanisms

The International Association for the Study of Pain defines pain as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage" (6). General categories include pain of nociceptive, inflammatory, and pathological mechanisms. Pain management strategies or treatments need to be targeted specifically depending on the type of pain present (7). Nociceptive pain is protective and the immediate response of the body that serves to limit contact with noxious stimuli by reflexive withdrawal in an effort to constrain tissue damage. Nociception is the "neural process of encoding and processing noxious stimuli" (8). Afferent sensory fibres have cell bodies located within the dorsal root ganglion. Sensory input from the periphery is transmitted through the dorsal nerve root into the dorsal horn of the spinal cord where it synapses with interneurons and is relayed to the sensory cortex via the ascending spinal cord tracts (9). Inflammatory pain is characterized by hypersensitivity of injured, inflamed tissues caused by stimulation of the local immune system. Inflammatory pain is also protective in that it limits joint or soft tissue movement or contact with the affected area allowing healing. In contrast, pathologic pain serves no biologic advantage (7) and often induces chronic or maladaptive pain that persists well beyond the presence of the initiating stimulus (10). Pathological pain is

categorized as neuropathic when there is direct injury to the nervous tissues, or dysfunctional when there are no organic lesions of the nervous tissues (7). Neuropathic pain has been described in horses with laminitis (11), trigeminal-mediated headshaking (12), and has been proposed as a component of the pain associated with osteoarthritis (OA) (13). It is possible that horses demonstrating cervical pain could be experiencing any of these types of pain, or possibly a combination of pain types.

3. Cervical dysfunction

Dysfunction simply implies impaired or abnormal functioning (14). Clinical signs of cervical dysfunction in human patients include decreased range of motion, altered body awareness and muscle activity (15), and has been described in equine patients with subtle gait abnormalities as well as abnormal muscle tone (16). In horses presented for poor performance, dysfunction is a critical, yet infrequently used term. Signs of cervical dysfunction may include regional or generalized muscle asymmetry (e.g., atrophy, hypertrophy, and hypertonicity), stiffness or inability to move the neck through a normal range of motion, and altered head or neck carriage (16). Cervical dysfunction may contribute to altered gait and biomechanics of the forelimb and trunk, which can produce additional dysfunction, pain and lameness.

4. Manifestations of cervical pain and dysfunction

4.1. Clinical Presentation

There is a wide-spectrum of clinical signs associated with cervical pain and dysfunction. Horses with cervical pain display obvious discomfort associated with palpation or active neck movements in work, as well as during stretching exercises or even daily routines. In contrast,

horses that have cervical dysfunction, without overt pain, may display more subtle signs of avoidance, it is possible for horses to display combined signs of pain and dysfunction. Affected horses in either category may have a history of a general decline in performance, neck pain and stiffness, an unwillingness to work on the bit, subtle hind limb gait abnormalities and lack of impulsion (17) and possibly forelimb lameness (18).

Horses with cervical dysfunction are often simply stiff or unwilling to be soft in the bend of their neck and body, may have difficulty with performing certain movements such as smaller circles, or they may pull against the reins or start tossing their head. While some horses are presented for a decline in performance or resisting work, other horses are more dramatic in their presentation. These horses may stop and refuse to go forward and may even rear and flip over backwards if the rider continues to ask in more forceful ways. Cervical radiculopathy in humans is a neurologic disorder that results from nerve root dysfunction either from mechanical compression or from local inflammatory mediators, and may result in myelopathy and muscle weakness (19). In horses, cervical radiculopathy typically results in localized pain within caudal cervical region and forelimb lameness due to peripheral nerve contributions from the brachial plexus (20). An unexplained change in behaviour is another common clinical sign recognized in horses with cervical pain or dysfunction. These horses display sudden onset of spooking within familiar surroundings or they are reported to act fearful (21). Riders and trainers may not always recognize these subtle behavioural changes, which may only be identified while acquiring a detailed history or be seen during on-site or ridden examinations. It is also possible for affected horses to develop apparent hypersensitivity whereby they resist being saddled, brushed, or even touched. Sometimes these horses even avoid the typical social greeting at the stall door or being

caught. Occasionally, horses are presented for concerns that seem unrelated to cervical pain, such as weight loss seen in horses with cervical pain that precludes them from reaching food on the ground or requires twisting their head to eat from a feeder. With many different clinical presentations, the practitioner must use detailed observation and all other forms of clinical information available to arrive at a diagnosis of neck pain and dysfunction.

4.2. Observation

When evaluating horses with neck pain and dysfunction, clinicians should perform a detailed, multistep examination and be careful not to overlook any perceived subtleties. Observing the natural behaviour of horses in their home environment is helpful as the stress and excitement of being in unfamiliar surroundings will cause some horses to override signs of pain and discomfort. Behavioural assessment is an important part of the overall examination to take note of (22). Careful attention should be paid to the horse's stance, facial expressions, and how the horse interacts with its surroundings and humans. The established horse grimace scale is helpful in assessing signs of pain through changes in facial expressions, such as subtle eye, ear, or mouth positions and characteristics (23). Assessing neck posture at rest relative to the position of head, limbs and trunk may help identify abnormalities (17); for example, horses with caudal cervical pain may shift their weight or alternate their forelimb placement from a normal square stance to a position with one limb predominately protracted and the other retracted. When encouraged to come to the stall door, horses may keep their necks in a very extended and rigid posture and only move their eyes to look toward the handler. Observing regional and left-right differences in muscle symmetry and development can help identify areas of muscle atrophy or hypertrophy that

may indicate underlying disuse or neurogenic muscle atrophy (24). Abnormal sweat patterns along the lateral cervical region may also indicate signs of sympathetic dysfunction (25).

4.3. Digital Palpation

Systematic and detailed palpation of the soft tissues and bony landmarks within the cervical region is a critical step in identifying and localizing potential sources of neck pain and dysfunction (e.g., stiffness, muscle hypertonicity). Soft tissues should be palpated from superficial-to-deep with specific focus on assessing texture and tone changes within the different tissue layers. Starting at the poll and continuing along the dorsal, lateral and ventral regions of the neck, the quantity (e.g., size or area) and the quality (e.g., severity) of heat or swelling is noted, which may be indicative of inflammation. The fascia is examined for tone and signs of tightness or reactions to the initial movement of the skin. The skin should glide freely in craniocaudal and dorsoventral directions over the underlying muscles without any signs of resistance or pain. Muscle tone and symmetry, and myofascial trigger points, which are discrete, painful, taut bands within the muscle that generate a referred pain pattern, are evaluated (26). Muscle development is assessed by examining the surface contours of the lateral cervical region and identifying regions of convexity (i.e., well-developed), flat (i.e., lack of development), or concavity (i.e., muscle atrophy). A common site of muscle atrophy or lack of development can be identified within the lower cervical region (C4-C6) affecting the splenius and semispinalis muscles. The brachiocephalic muscles are commonly painful on manual compression in horses with lower cervical pain or stiffness, which can be identified with firm compression of the muscle beginning at the upper and progressing to the lower cervical region. Finally, an avoidance

response after deep palpation over the cervical transverse processes and articular processes might indicate the presence of bone or joint pain.

4.4. Dynamic Spinal Examination

For the dynamic spinal examination, both passive and active spinal movements are analysed. Passive spinal movements are applied to assess joint and soft tissue movement without muscle activation; whereas, active spinal movements consist of the patient initiating the motion, which requires muscle activation (27). In order to be as specific as possible with respect to the individual vertebrae affected in normal or dysfunctional cervical motion, active exercises are performed with the horse placed against a wall or in a corner, so it does not step away when being asked to perform the specified movements (17). It is important to evaluate lateral bending and the ability to flex and extend the entire head and neck region from both quantitative (e.g., range of motion) and qualitative (e.g., ease and fluidity) perspectives. Some horses resist some or all of these movements, which may relate to pain stemming from individual articulations or due to overall stiffness or muscle guarding throughout the entire neck. Treats may be used to encourage neck movement for active mobilization; however, some horses are not well motivated by food, while others may aggressively lunge for the treats. Horses with normal neck mobility can readily move their heads and necks from side to side and position their chin near the girth, hip, or tarsus (28). When asking the horse to go through these lateral bending movements, most of the mobility occurs within the cranial and caudal cervical regions, with less mobility noted at C2-C5 (28). Some horses will quickly rotate their heads axially at the atlantoaxial articulation when asked for lateral bending and are unable to bend in the middle or lower cervical regions due to stiffness or pain. If a horse is painful and unable to readily laterally bend its neck, then

repeating the motion while keeping the chin further away from the body by approximately 30cm may be helpful (28). Evaluating flexion and extension is used to assess mobility at the atlantooccipital articulation and lower cervical region. These exercises include asking the horses to extend their nose out in front of them and elevating it as high as possible. Flexion exercises typically include movements along the mid-sagittal plane where the nose is brought toward the point of chest, carpus and fetlock region (29).

4.5. Gait Examination

Horses with cervical pain and dysfunction should undergo a thorough evaluation of gait, including assessment for lameness and neurologic disease. The axial skeleton can play an important role producing subtle gait abnormalities, which might be initially missed by riders, trainers, and veterinarians who are focused on issues affecting the appendicular skeleton. Routine lameness examination is aimed at identifying gait abnormalities stemming from appendicular musculoskeletal disease; however, it is important to recognize that careful static and dynamic examination of the axial skeleton is necessary to assess axial-appendicular interactions and possible compensatory gait mechanisms. In addition to the routine lameness examination performed at the trot on a straight line and circles, it is beneficial to examine affected horses under tack at different gaits. Abnormalities in head or neck carriage may only be appreciated on the lunge line with the head carried toward the outside of the circle (i.e., difficult to turn in one direction) or gait deficits noticed with or without using side reins. Other horses may have pronounced lowering of the head and extension through the lower cervical region and appear to have generalized neck stiffness. Horses may compensate for cervical pain or dysfunction by changing forelimb flight patterns (e.g., hopping-like motion) or by asymmetrical gait patterns

due to cervical radiculopathy (20, 30). Some affected horses appear to have an apparent weakness or difficulty in fully engaging the forelimbs in the early stance phase, which may precipitate stumbling. Forelimb lameness that cannot be localized to the limb with diagnostic analgesia may originate from the cervical region. Intraarticular (IA) analgesia of the (APJ) can be considered in select cases to help confirm cervical localization of pain and inflammation as the source of lameness (20, 30, 31). Horses that are generally stiff and resistant, however, do not seem to be good candidates for IA analgesia of the APJs as it is often difficult to gauge a level of improvement due to the high likelihood of multiple vertebral levels being affected.

A complete neurologic examination should also be performed to determine whether neurologic disease could be contributing to the observed clinical signs. Specifically, diseases such as cervical vertebral compressive myelopathy (CVCM), equine degenerative myelopathy, and equine protozoal myeloencephalitis should be considered. Ancillary diagnostics such as cervical myelography and serum and CSF SAG2,4/3 antibody testing can be pursued, as indicated (32). Further, the presence of underlying myopathies including polysaccharide storage myopathy, vitamin E-related myopathies, and myofibrillar myopathies should be considered. An exercise challenge with evaluation of muscle enzymes and muscle biopsies can be pursued if indicated (33).

5. Osseous Sources of Cervical Pain and Dysfunction

5.1. Cervical Articular Process Joint

Synovitis and associated joint pain are commonly diagnosed as a cause of poor performance in horses (17, 18). The APJs contribute to the “spinal motion unit”, which consists of the two

dorsally-paired diarthrodial articulations and the ventral intervertebral disc. In horses, two commonly noted abnormalities affecting the APJs are osteochondrosis (OC) and OA. Osteochondrotic lesions vary from small fissures within the articular surface to large irregularities with evidence of secondary OA (34). OC diagnosed via imaging and histopathology has been noted in young horses with CVCM (35). Horses with OC of the cervical articular surfaces, as is true of the appendicular skeleton, may have subtle to no clinical signs associated with the lesions. Other horses, however, may experience progressive inflammation and pain of the APJ secondary to OC (36).

OA is a disease of the cartilage surface and bone structure; however, it is important to recognize that other structures within the joint complex, which include subchondral bone, joint capsule, synovium, and paraspinal muscles, are also affected and can be a primary source of cervical pain (37). Radiographic evidence of OA of the APJ includes joint enlargement, subchondral sclerosis, extension of the dorsal laminae, joint margin lipping, and the presence of osteophytes and joint capsule enthesophytes (38). A recent post mortem study in a mixed population of horses showed that the most commonly noted bony changes on the caudal articular processes were modelling and joint margin flattening, while the most common changes noted on the cranial articular processes were osteophyte formation, joint margin lipping and enthesophytes of the joint capsule with the most severe changes noted at C2-C3 and at C7-T1 (37). Interestingly, a retrospective study found that the C5-C6 APJs are enlarged with no correlation to clinical signs (39) and it has been reported that approximately 50% of normal mature horses have some degree of unilateral or bilateral changes in cervical joint margins (39). These findings make it impossible to diagnose

cervical pain or dysfunction solely based on radiographic images and caution should be used to avoid the over-interpretation of OA as the primary cause of the observed clinical signs.

5.2. Vertebral Body

Morphologic variations affecting the vertebrae have been documented extending from the occiput (40) to the cranial thoracic region (41). Of particular interest in performance horses are malformations affecting the caudal cervical vertebrae, which may include unilateral or bilateral transposition of the ventral process from C6, variation in the size and shape of the C7 spinous processes, and the presence of additional ribs or costal processes (41). Improvements in diagnostic imaging techniques provide better visualization of these areas (42, 43), which will ultimately allow for an increased understanding of the clinical significance of these malformations. While the clinical significance has been questioned (44), horses with transitional anomalies of the C6 lamina have been reported to have an increase in perceived cervical pain and decreased joint range of motion, likely secondary to altered attachment of the longus thoracis muscle (45).

While fractures of the cervical vertebrae usually follow acute trauma (17), affected horses may also present with neck stiffness and poor performance without any known trauma. As indicated in any suspected fracture, radiography is a first-line imaging tool to confirm the diagnosis. In some cases, the fracture may not be easily identified and computed tomography (CT) may be indicated. Judicious use of non-steroidal anti-inflammatory drugs (NSAIDs) is indicated in acute neck pain cases along with confinement and management strategies such as offering food and water at a neutral head and neck level, which may help to minimize induced motion at the

fracture site (17). As healing progresses, it is important to monitor the comfort of the horse, amount of callous formation, and possible consequences to the APJs (e.g., osteoarthritis) and vertebral canal (e.g., CVCM). Moreover, it is possible for the vertebral segments cranial and caudal to the fracture site to be impacted by altered biomechanics of the neck. Prognosis is influenced by the structures involved in the fracture and healing process, the degree of instability at the fracture site, and the neurologic status of the horse.

6. Soft Tissue Sources of Cervical Pain and Dysfunction

6.1. Cervical Fascia

The fascia is connective tissue that surrounds and connects every muscle and organ, forming a continuous collagen network within the body. The superficial fascia is highly vascularized and innervated, while the deep fascia has a role in isolating individual muscles or muscle groups and providing attachment for muscles (46). In humans, the deep fascia has been found to be the most pain-sensitive tissue in the thoracolumbar region in both acute and chronic pain conditions (47). The deep fascia can undergo densification and fibrosis which changes the ability of the tissues to glide and alters the nerve fibre function, leading to an increased pain state (48). The fascia specific to the horse has recently been evaluated and found to be quite compact and tightly connected, likely related to the need for an energy-efficient system (49). In horses with cervical pain and dysfunction, compensatory nociceptive and biomechanical mechanisms likely contribute to densification of the fascial tissues and the development or chronic recurrence of pain.

6.2. Cervical Muscles

The cervical musculature is likely an important source of neck pain and dysfunction. The brachiocephalicus muscle is a superficial muscle that extends from the occipital bone to the humerus, while the adjacent omotransversarius muscle extends from the wing of the atlas and inserts on the cervical transverse processes and on the spine of the scapula. Both of these muscles serve a primary role in shoulder extension and forelimb protraction (50). If these muscles are painful or weak, horses may present with a decreased cranial phase of the stride and reluctance to laterally bend away from the affected muscle. The splenius muscle contributes to lateral bending of the head, prevents unwanted flexion of the head during movement, and provides static postural support (51). The dorsal portion of the semispinalis muscle imparts passive support to the head and neck; while the ventral region plays a more active role to raise the head (51). The splenius and semispinalis capitis muscles and nuchal ligament all function to resist gravitational forces and to actively elevate the head and extend the neck (52). The deep cervical musculature function in stabilizing individual cervical intervertebral joints and consist of dorsal, lateral and ventral muscle groups, which include the multifidus, intertransversarius and longus colli muscles, respectively (37, 52). The multifidus cervicis is a complex muscle with multiple intersegmental fascicles from the level of C2 through the cervicothoracic junction (52). The fascicles of the multifidus have caudal attachments to the joint capsules of the APJ (53) and proper function of these stabilizing muscles is important for neuromotor control, proprioception and joint stability. In humans, atrophy or weakness of the multifidus and longus colli muscles are often associated with whiplash injuries and neck pain(54). Segmental atrophy of the multifidus muscle has also been observed in horses associated with APJ pathology (52, 55). The longus colli muscle also has distinct intersegmental fascicles from C1 through C5. At the level of C6-

T5, this multi-fascicular structure is replaced by a single muscle belly to form the longus thoracis muscle which attaches to the caudal C6 transverse (52). Variations of attachment sites of the longus colli, secondary to developmental anomalies of C6, have been shown to be associated with proprioceptive and neurologic dysfunction (56). This highlights the importance of anatomical features, articular pathology, and functional control of the cervical muscles to prevent or limit the development of neck pain and dysfunction.

6.3. Nuchal Ligament Desmopathy

The nuchal ligament is a bilobed, highly elastic structure that connects the occiput and cervical vertebra to the cranial thoracic spinous processes. Occipital enthesophytes have been reported in 85% of 302 warmblood horses aged 1-22 years (17). Horses with suspicion of nuchal ligament enthesopathy or desmopathy may be unwilling to position their head straight in the bridle, resist poll flexion, and may have inconsistent responses to soft tissue palpation (17). Radiography and ultrasonography are indicated to evaluate the attachment sites of the semispinalis tendon along the caudal occiput and the fibre pattern and attachment of the nuchal ligament. Local anaesthetic infiltration may be warranted to determine the clinical significance of positive radiographic findings and the response to ridden exercise and induced poll flexion. Caution is needed to avoid the occipitoatlantal epidural space during local anaesthetic infiltration to avoid inducing ataxia (17). Treatment trials with acupuncture, spinal mobilization, laser therapy, and extracorporeal shockwave therapy (ESWT) may help to improve the clinical signs associated with enthesopathies in this location. Surprisingly, horses that avoid palpation of the poll region seem to respond well to ESWT and generally require minimal sedation. Using a piece of felt under the

crown-piece of the bridle, or using a headstall designed to redirect pressure more caudally over the poll region may also reduce the clinical signs in some horses.

6.4. Nuchal Bursitis

Bursal inflammation can stem from infectious and non-infectious causes that result in pain, stiffness, and abnormal head and neck postures. The funicular portion of the nuchal ligament travels over the dorsal aspects of the atlas (C1) and axis (C2) as it attaches to the caudal aspect of the occiput. The cranial (dorsal to C1) and caudal (dorsal to C2) nuchal bursae are potential spaces within the fascial layers and are not readily identifiable unless filled with fluid. Localized swelling can sometimes be palpable, but a more definitive diagnosis is possible with ultrasonography (57). Therapeutic options include medical therapy (i.e. rest, NSAIDs with or without intrabursal treatment), as well as surgical debridement, both may be curative in some horses (58).

6.5. Cervical Joint Capsule Fibrosis and Synovitis

Stretching or injury to the APJ capsule is considered an important source of cervical pain in humans (9, 59) and goats (60). A high density of A-delta and C fibre receptors within the joint capsule have nociceptive as well as proprioceptive roles (61). Capsular microdamage is capable of evoking pain through sustained nociceptive firing (62). As the multifidus muscle attaches directly to the cervical joint capsule in both humans and horses, dysfunction of the multifidus cervicis muscle and subsequent altered cervical biomechanics with increased forces applied to the joint capsule, may play an important role in the etiopathogenesis of equine cervical pain (52). Synovitis may also be a significant source of neck pain through pressure from increased joint

effusion and inflammatory mediators (e.g. metalloproteinases, prostaglandins, free radicals, and a number of cytokines including IL-1β and TNFα) (63).

6.6. Cervical Intervertebral Disc Disease

Intervertebral disc disease is common in humans (64) and dogs (65, 66). In horses, there are limited case reports mostly focusing on end-stage disc degeneration and vertebral endplate eburnation (67, 68). More recently, the earlier stages of intervertebral disk degeneration of horses have been reported (69, 70). In contrast to previous literature (71), the study by Bergmann suggests that the intervertebral disc has a grossly and histologically discernible proteoglycan-rich central region that is judged to be different from the lamellar collagenous annulus fibrosus (69). In this study, they proposed a grading scheme for intervertebral disc degeneration that had very good intra- and interobserver reliability which may be useful for future research. In a previous report of end stage disease (68), horses showed severe neurologic dysfunction including pelvic limb ataxia and recumbency. However, early stages of disc degeneration and segmental instability may produce subtle signs of spinal cord compression in addition to cervical pain and dysfunction and possible lameness (70).

7. Nervous System Structures in Neck Pain

7.1. Spinal Nerve Roots

Spinal nerve roots exit through the intervertebral foramen (IVF) and have been reported to be at risk of impingement (18) or possible radiculopathy secondary to severe OA of the APJ (72), similar to what is seen in humans. Although a recent publication characterizing bony changes of the APJ in horses (37) did not identify size limitations of the IVF to cause physical impingement,

and investigators were not able to create nerve root impingement after distending the APJ capsule (73), there appears to be clinical evidence to suggest nerve root impingement or a similar syndrome occurs in the horse (74). It is also likely that inflammatory mediators associated with APJ OA contribute to chemical-induced neuritis and the development of neck pain and dysfunction. This may be difficult to confirm in vivo, but histologic evidence of nerve root injury has been noted at post-mortem examination in horses with unexplained forelimb lameness (20). The in vivo diagnosis of cervical radiculopathy or neuritis is still presumptively based on collective clinical examination findings, radiographs suggestive of APJ arthropathy, electromyography, three-dimensional imaging, and exclusion of other causes of cervical pain and dysfunction. It is the authors' opinion that these horses are often quite painful and reactive, not just stiff and mildly resistant. Affected horses may be quite explosive and unpredictable in their reaction to being asked to bend and engage their cervical region. As has been described, horses may present for a forelimb lameness that cannot be localized, or with a "hopping-limb" forelimb lameness (20).

7.2. Dorsal Root Ganglia

The dorsal root ganglia (DRG) is a cluster of neuronal cell bodies located in the paraspinal area that contain the cell bodies of afferent sensory neurons. Their location, at or within the IVF, results in a potential increased risk of injury to the sensitive neuronal tissue. Unlike the central nervous system, there is no neurovascular barrier protecting the DRG. In the horse, ganglionitis has been described related to chronic laminitic pain (11, 75) and as a proposed mechanism of headshaking and trigeminal neuralgia (76). More recently, there has been identification of lymphocytic inflammation within or around the DRG in horses identified with "idiopathic

hopping-like forelimb lameness" (30). The clinical relevance of dorsal root ganglionitis and its relationship to equine chronic neck pain remains to be elucidated.

7.3. Spinal Cord

The most common cause of spinal cord disease is cervical spinal cord compression seen in CVCM (35, 77-79). While CVCM is a common condition in horses presented with varying degrees of symmetric ataxia, evidence of neck pain in this population of horses is not always present. In fact, young horses with developmental orthopaedic lesions causing stenosis of the vertebral canal often have no evidence of neck pain. In contrast, older horses with OA of the APJ may have ataxia and have a stiff and painful neck on examination (80), or are presented for neck pain and are found to have subtle ataxia on clinical examination.

8. Diagnostic Imaging

8.1. Radiography

Radiographs provide a good baseline screening tool for horses with neck pain or dysfunction; however, it is important to recognize that some cervical lesions are radiographically occult and that some findings, such as enlargement of the APJs, may not be clinically significant (24, 39). Radiographs are indicated in horses with a history of cervical trauma, neck pain or stiffness, decreased performance, gait abnormalities associated with neurologic deficits, or forelimb lameness that is not readily localized to the limb. Lateral-lateral radiographs from the caudal skull to the first thoracic vertebrae are readily acquired in the standing, sedated horse to allow evaluation of bones, including morphology and alignment. Radiographs also provide indirect evidence of soft tissue injury, including the presence of spinal cord compression if there is

obvious vertebral canal stenosis. The technique for acquiring oblique images of the cervical vertebrae has been described (81) and can improve detection and better localization of bone pathology (82). The clinical significance of radiographic abnormalities may require further investigation, such as the response to IA analgesia or treatment trial. All radiographic findings must be interpreted in conjunction with the clinical examination findings.

8.2. Ultrasonography

Cervical ultrasonography is frequently used as an adjunct to radiographs to further assess changes in cervical APJ margins, joint capsules, and other soft tissues (e.g., nuchal ligament and bursae). A complete examination of the cervical region extends from the caudal occiput to the last visible articulation, typically C7-T1 (82). Changes of the APJs that can be identified include periarticular bone remodelling and osteophytes, increased joint fluid, and thickened joint capsules. Mild periarticular osteophytes in the absence of joint effusion or capsular change are more difficult to interpret as the significance of this finding is variable. Joint effusion is rarely found incidentally and suggests an active joint disease or inflammation. Accurate evaluation of the IVF and spinal nerve roots is limited due to the oblique angle of the ultrasound probe relative to the underlying anatomy. Cervical ultrasound is not only used for diagnostic purposes, but also to guide administration of medications or anaesthetics into the synovial articulations (83-85).

8.3. Nuclear Scintigraphy

Nuclear scintigraphy is a commonly used tool for the diagnosis of obscure lameness and poor performance in horses; however, it is a relatively insensitive technique in the cervical spine and may produce false-positive results (17). The normally larger size of the C6-C7 APJ is associated

with greater uptake than adjacent articular processes, which can be overinterpreted as a significant finding. Additionally, enlargement and remodelling of the APJs can result in increased uptake with no clinical significance (86). False negative results are also possible, particularly in the caudal neck, due to the thick overlying muscle mass and scapular shielding (17). Negative findings on scintigraphy do not rule out pain originating from the cervical vertebrae, rather merely rules out the presence of increased bone turnover. Appropriate image acquisition requires obtaining both left and right lateral images for accurate lesion localization and to better direct therapy (82). As with all cervical imaging, results must be correlated with other clinical examination and imaging findings.

8.4. Three-dimensional Diagnostic Imaging

Advanced imaging of the cervical spine includes CT, CT myelography, and magnetic resonance imaging (MRI) which has been used to describe normal cervical anatomy (87). MRI is a non-invasive method to evaluate the cervical nerve roots, but at this time it is not capable of imaging the caudal cervical spine in most adult horses (20). With increased attention to cervical disease in the horse, the demand for magnet configurations that allow for examination of the entire cervical region may increase the viability of MRI to be used in ante-mortem assessment of the cervical spine, including the IVF and cervical nerve roots. While MRI is the gold standard for cervical imaging, currently CT is more clinically available for imaging the entire length of the equine cervical region. CT provides excellent bone detail and allows for more comprehensive assessment of osseous changes compared to radiographs. For example, CT allows for determination of orientation of APJ enlargement and identification of medial versus lateral extension of the joint margins onto the spinal cord and spinal nerve roots exiting the IVF.

Contrast-enhanced CT widens its diagnostic applicability, and can be utilized in whole body, IA, or intra-thecal applications. IA contrast helps define the APJ, especially the articular cartilage surface. CT myelograms are commonly indicated to diagnose CVCM (74) as well as having applications in diagnosing OA of the APJ, fractures, malformations and some soft tissue lesions (88). However, CT lacks the soft tissue detail to assess neuritis, myositis, and early intervertebral disc degeneration amongst other soft tissue injuries; MRI is the optimal modality to assess such soft tissue changes.

8.5. Electrodiagnostic Evaluation

Electromyography is used to directly assess the neurophysiologic status of the motor unit and its individual constituents, including the alpha motor neuron, its axon, the motor endplates and the associated muscle fibres (89, 90). This technique is used to differentiate between neurogenic and myogenic disorders, and can provide insight into severity and distribution of lesions. Determination of nerve conduction velocity can provide further insight into function of peripheral nerves. However, proper data collection and interpretation requires a thorough understanding of neuromuscular physiology and associated technical factors (90). Possible indications include chronic, poorly localized lameness and neurologic dysfunction from unknown causes (91).

8.6. Surgical Evaluation

Epiduroscopy is a technique that has been described that allows direct visual inspection of the dorsal and ventral nerve roots to the level of the 8th cervical nerve (92). This technique is in early experimental stages and is not routinely used clinically. Needle scope arthroscopy has recently

been described (93) which has the potential to allow a more complete evaluation of the APJ in a standing horse. Both of these techniques need further exploration into the potential benefits they may offer to the understanding of cervical pain or dysfunction.

9. Treatment Options

9.1. Systemic Medications

9.1.1. Non-steroidal anti-inflammatory drugs (NSAIDs)

NSAIDs are the most frequently used analgesics in horses worldwide (94). When using NSAIDs for treating cervical pain, drug toxicity, drug doses and competition rules should be considered (95). NSAIDs may not have the desired efficacy in treatment of some affected horses; it is possible that the anti-inflammatory action of NSAIDs is ineffective in pathologic pain conditions, which have no primary inflammatory component. However, in acute injuries with inflammation, NSAIDs are indicated. Long-term dosing with firocoxib may be helpful in some chronic cases of cervical OA. However, it is the authors' opinion that the complexity of cervical pain and the possibility of a neuropathic pain component makes this medication less rewarding than may be noted when used for appendicular skeleton lameness.

9.1.2. Bisphosphonates

Bisphosphonates have reported effects on bone turnover and therefore may have therapeutic effects to alter the remodelling phase in certain types of bone pathology. Clodronate and tiludronate have been approved in the United States for treatment of navicular disease in the horse (96). Tiludronate has been reported to improve flexibility in horses with clinical and radiographic findings suggestive of OA of the APJ within the thoracolumbar region (97). In addition to bone-sparing properties of bisphosphonates, anti-inflammatory effects occur via

decreased nitric oxide and cytokines released from activated macrophages (98). These mechanisms of action make this class of drug a reasonable consideration in horses that present with neck pain and radiographic evidence of bone remodelling when improvement of clinical signs of cervical pain has not been achieved in more commonly used therapies.

9.1.3. Gabapentin

Gabapentin is an antiepileptic agent that is commonly used to treat neuropathic pain in veterinary patients (99) and as a first line treatment in humans (100). Gabapentin acts on the voltage-gated calcium channels localized primarily at the synapses (101), decreasing neuronal excitability through binding of the $\alpha_2\delta$-1ligand and altering pain processing (99). In rats, gabapentin in combination with either diclofenac or celecoxib has higher efficacy and safety than either drug alone (102). There are no studies in horses evaluating this synergy, so it is unknown if combination therapy with NSAIDs is necessary for improved efficacy. Gabapentin was not associated with any negative cardiovascular or behavioural effects but was shown to have a low bioavailability of 16% with oral dosing at 20mg/kg in horses (103). Gabapentin may be considered as a reasonable treatment in horses with neuropathic or chronic neck pain. Clinically, this is a medication to consider in very painful or hypersensitive horses.

9.1.4. Muscle Relaxants

Muscle relaxants may be used alone or in combination with other medications or modalities such as Extracorporeal Shockwave Therapy (ESWT) when the horse is experiencing hypertonic or painful cervical muscles. While the author does not use muscle relaxants routinely as a first-tier treatment, these drugs could be considered for certain cases, such as a horse with a very tight,

reactive brachiocephalicus muscle. These horses may have a shortened cranial phase of the stride and be unable to retract the limb comfortably. The author has found that these medications can bring some relief in such instances. Methocarbamol is a centrally acting skeletal muscle relaxant, labelled to reduce muscular spasms (104), and is commonly used as a first-choice drug for these cases. There is a very large dose range for intravenous use of 4.4-55.0 mg/kg. There is not an FDA-approved label dose for oral use; however, it has been reported to be used at 2-3 times the IV dose (105). Dantrolene, another skeletal muscle relaxant used in horses, acts by suppressing calcium release, subsequently interfering with excitation-contraction coupling in the muscle fibre (106). There is no FDA labelled product for use in horses, although the human product may be used off-label, and a dose of 4 mg/kg has been published and shown to decrease serum Creatine kinase (CK) after exercise (107).

9.2. Physical Medicine

9.2.1. Chiropractic

Chiropractic in horses is more and more commonly implemented for back pain (108) and there is evidence to support the beneficial effects (109). While the same has not been reported for equine cervical pain, the physiology of chiropractic medicine supports implementing this therapy for cervical pain and dysfunction. Chiropractic, a form of manual therapy, uses high-velocity, low-amplitude thrusts, specifically aimed at the joint (108). Chiropractic medicine may be implemented as both a diagnostic or therapeutic modality, with the goal of treatment being to restore normal joint motion, reducing pain, and stimulating neurologic reflexes (110). Hypomobility of a joint may give rise to a number of problems, including muscle spasm, nerve dysfunction, and pain (111). Horses with chronic neck pain or dysfunction often show

compensatory changes in their biomechanics which subsequently predisposes them to further injury (108). Spinal mobilization is indicated for neck or back pain, localized or regional joint stiffness, and commonly for poor performance or asymmetric gait without overt lameness (27). In human patients, chiropractic is commonly used for management of neck pain. Additionally, chiropractic in humans has been shown to improve proprioceptive input from the cervical spine, which could affect postural control as well as decreasing pain (112). Contraindications of chiropractic include fractures, neoplasia, spinal instability and acute conditions that require more conventional therapy. Therefore, having a thorough understanding of any underlying pathology or disease state is of utmost importance when implementing chiropractic medicine in horses with neck pain and dysfunction.

9.2.2. Therapeutic Exercise

While performing dynamic exercises of the cervical spine, employing concentric muscle activation, the horse's posture is altered. Stability of the body and limbs is then achieved through activation of the abdominal, epaxial, and pelvic muscles, through isometric or eccentric actions (29). Performed in the same fashion as was described in the dynamic spinal examination section, the mobilization exercises aimed at the cervical spine not only mobilize the joints in the cervical region, but may also activate and strengthen both the epaxial and hypaxial muscles along the entire axial skeleton. This whole-body activation and strengthening may change the functional movement patters and neuromotor control (29). Additionally, when performing dynamic exercises, the range of motion is controlled by the horse which decreases the risk of the joint moving out of the comfort zone, as is the case in passive stretching. There is a more normal neuromotor control when stimulating the muscles that move and stabilize a specific joint (29).

Exercise therapies are critical for the effective management of human patients with neck pain (113). In humans, the longus colli and longus capitis muscles (i.e., deep cervical flexors) have an important role in joint support and control, which cannot be reproduced by the more superficial muscles (114). In horses, the multifidus cervicis and longus colli muscles also function to provide dynamic segmental stability and support in the cervical spine (52). When developing a rehabilitation program to improve joint stability, focusing on these deep paravertebral muscles is an important consideration. As with all rehabilitation protocols, it is important to have a working knowledge of the related anatomy and what tissues are believed to be compromised so that a program can be tailored specifically on a horse-by-horse basis.

9.2.3. Acupuncture

Acupuncture is used to stimulate nerves, muscles, and connective tissues throughout the body with the goal of alleviating pain (115). As a general concept, it is believed that the insertion of the needle into the skin and manipulation of the tissue with manual acupuncture, or stimulation with electrical currents with electrical acupuncture causes a number of reactions locally, as well as at the level of the spine, and in the brain (116). Altering the neural activity in response to acupuncture causes the synthesis and release of neuromodulators that have the potential to have a therapeutic effect in many disease states (117). Neuromodulation with acupuncture is used to control pain and inflammation in humans (116), and likely has a similar physiologic response in horses. In rodent models, it has been shown that acupuncture may protect against articular cartilage erosion (118) as well as chondrocyte inflammation (119), and these effects may help in clinical cases of cervical OA. It is the authors' opinion that many horses experiencing neck pain

respond well to acupuncture of the cervical region and acupuncture may help prolong treatment intervals when used in conjunction with other therapies.

9.2.4. Mesotherapy

Mesotherapy is a minimally invasive technique where small doses of medications are given intradermally in regions of musculoskeletal pain. Mesotherapy is used for many conditions in human medicine, with pain management being a primary justification (120). Common medications used for injection include local anaesthetics, corticosteroids or saline in order to disrupt the local pain reflex arc (55). In equine practice, mesotherapy is thought to be useful in the treatment of neck pain, reducing muscle spasm and improving the range of motion in horses with chronic neck pain (55). For practitioners trained in acupuncture, mesotherapy is likely less frequently utilized, as some of the pain inhibition pathways are quite similar.

9.2.5. Electrotherapy

There are multiple forms of electrotherapy that may be used for pain management. Some of the more common modalities utilized include transcutaneous nerve stimulation (TENS), pulsed electromagnetic field therapy (PEMF), and neuromuscular electrical stimulation (NMES). It is believed that TENS therapy modulates pain through activation of the descending inhibitory system as well as by increased release of endogenous opioids (121). Although there is limited research to support using TENS therapy for pain relief in horses (122), there is data in humans with knee OA to show a beneficial effect on knee pain (123). PEMF therapy uses electromagnetic fields to produce secondary electrical currents in a tissue and has been used to provide pain relief as well as improving function in humans with OA (124). In a study in ponies

with induced carpal synovitis, PEMF treatment showed a positive effect (125). There are multiple ways to employ PEMF including coils, blankets and wraps (122). NMES may be used for muscle development and stimulating neuromuscular control (126) by causing depolarization of a motor neuron. A specific type of NMES used in horses is a functional electrical stimulation (FES) unit (122), which has been shown to improve functional movement and decrease epaxial muscle spasm in horses (127). While this is encouraging, the literature in humans is inconsistent, and some horses may be apprehensive of this type of therapy (126).

9.2.6. Extracorporeal Shockwave Therapy

The mechanism of action of ESWT is not fully understood; however, there is good evidence of an immediate effect of this technique on pain receptor physiology as well as initiation of fascial tissue healing (128). The use of ESWT for horses was initially adapted from human medicine where a positive effect for treating insertional desmopathies was found (129). ESWT is commonly utilized for treatment of horses with nuchal ligament desmopathy with reported positive results (129). ESWT has also been shown to decrease pain and improve cervical range of motion in human patients with myofascial pain syndrome (130). ESWT has recently been shown to raise the mechanical nociceptive threshold in horses with back pain (131). Anecdotal evidence suggests that horses with myofascial pain and restricted mobility of the cervical spine also respond favourably to ESWT. In addition to the soft tissues that may respond favourably, ESWT has been shown to be beneficial in the treatment of osteoarthritic conditions in human and veterinary medicine (132, 133) and therefore may also help horses with cervical OA.

9.2.7. Elastic Therapeutic Tape

Elastic therapeutic tape is used to increase local circulation and therefore reduce oedema, give stimulation to the skin, muscle or fascia and provide afferent input to the central nervous system (134). In human medicine, there is limited data to support short term pain relief and cervical range of motion with elastic therapeutic tape (135). In equine practice, the use of elastic therapeutic tape has become quite common, with applications from the competition ground to rehabilitation facilities (136). While the use of therapeutic taping is growing, it is important to recognize the mechanisms of action and possible beneficial outcomes remain unclear. While there is some thought that tape can have pain-relieving effects, therapeutic taping is more often used for equine cervical dysfunction.

9.3. Local Therapies

9.3.1. Intra-articular corticosteroids

While many horses will respond positively to less invasive management strategies, horses with clinical signs and imaging findings consistent with cervical OA frequently benefit from IA application of corticosteroids, which is commonly performed in horses with cervical pain (137). Birmingham reported that 71% of symptomatic horses returned to normal function or improved in performance after cervical IA corticosteroid treatment (137). One limitation of the study was inconsistency in the dosage and type of corticosteroid used, as well as treatment frequency and concurrent therapies provided. Similarly, in clinical practice there are regional and personal differences in the type of corticosteroid used, frequency of treatment and other concurrent therapies. Ultrasound-guided injection of cervical APJ is a well-established technique that is easy to perform (83), (84). However, success is heavily based on operator experience (83). While the procedure itself requires the use of ultrasound guidance for appropriate administration of the

medication, many of the same considerations regarding what medication to use, frequency of administration, and return to work are similar when treating the cervical APJ as for any other IA therapy in a high-motion joint.

9.3.2. Biologic Therapies

Biologic therapies could be considered as an alternative to corticosteroids when the metabolic condition (e.g., insulin resistance) suggests the horse be at an increased risk of laminitis or when medication rules and withholding time would disallow the use of IA corticosteroids. Autologous conditioned serum (ACS) is commonly used to inhibit the effects of IL-1β (138). In the appendicular skeleton, once-weekly ACS therapy for 3-4 treatments is a commonly used protocol (139); however, there are no standard treatment schedules for using ACS in cervical APJs, and if treating multiple joints of the cervical spine, this protocol can become cost-prohibitive. Anecdotally, the author has seen a positive response in a small number of horses treated with IA administered ACS in the cervical APJs. In humans with lumbar radiculopathy, ACS and triamcinolone both suppressed pain and disability, but ACS was potentially superior to triamcinolone for long-term pain relief (140). There is anecdotal evidence to support the use of stem cell therapy for OA in other articular locations. However, to the authors' knowledge there are no reports of equine cervical arthropathy cases successfully treated with stem cells at this stage. Further information is warranted before adopting this treatment approach.

9.4 Surgical Therapies

9.4.1 Arthroscopy

Arthroscopic evaluation of the APJ may be indicated for diagnostic as well as therapeutic purposes. Surgical considerations could include removal of OC fragments or a better understanding of the cartilage health and integrity. A technique has been described for a lateral approach to the cervical articulation (141). While the procedure was successfully performed, two of the three horses were euthanized within 96 hours of the surgery due to poor prognosis and severe clinical presentation. A more recent report by Tucker et al. describes arthroscopic removal of an OC lesion from C4-C5 in which there was an initial positive response to surgery, but at six weeks post-operatively acute neurologic signs required humane euthanasia (142). Improvements to the technique and better case selection criteria are necessary before this technique becomes common practice.

9.4.2 Cervical Vertebral Stabilization

While cervical vertebral stabilization has typically been considered only to benefit horses with CVCM and cervical fractures, there is increasing evidence for this procedure to benefit horses with other neck disorders that result in conditions such as neck pain, stiffness, or radiculopathy (143, 144). Indeed, regression of bony arthritic changes was seen 12 months post-operatively with all horses showing resolution of neurologic signs (145). Two common surgical procedures for cervical stabilization are the use of a Bagby basket (146, 147) or a kerf-cut cylinder (148). A more recent method being applied is with a polyaxial pedicle screw and rod construct (149). All of these procedures are technically demanding and should be performed only by those with a solid understanding of the cervical anatomy and a high level of surgical training. As imaging modalities continue to improve and evaluation of the cervical spine in multiple planes is possible, we gain a broader understanding of the structures of the cervical spine. For example,

altered articular process shape, size and spatial positioning has been shown to result in compression of the spinal cord from the dorsolateral aspect (150). It is important to continue utilizing our creativity and exploring novel therapeutic options for horses with cervical disease.

10. Conclusions and Future Directions

In horses presented for declining performance or behavioural issues, it is of paramount importance to first determine if, and possibly what type, of pain the horse may be experiencing. On the surface this seems like an easy task; however, the diagnosis of cervical pain is not always straightforward, and the clinician must consider all available information: history, observation, static palpation, motion palpation and dynamic evaluation. It is the authors' opinion that the most critical component moving forward in order to answer this question is appreciating the myofascial examination. The body, and our ability as clinicians to interpret the signs the horse is telling us, must be acknowledged first and foremost. As practitioners we must develop a systematic approach to the myofascial examination and watch for the subtle signs from the horse. While this seems straight forward, the interpretation of the examination, and fitting it to the clinical picture is complex. We must acknowledge that the reactions are frequently not simply behavioural problems. Diagnostic imaging is indicated to help identify or localize the affected tissue or structures as possible sources of pain, recognizing that all modalities have strengths and limitations, and a clear diagnosis may still not be readily obtained. Looking ahead, the development of imaging modalities capable of evaluation of the cervical region is critical. Computed Tomography (CT) and Magnetic Resonance Imaging (MRI) technology is improving and beginning to allow these examinations to be performed on a more clinical basis. Our

understanding of the underlying disease process needs to advance concurrent with our ability to image these areas so that we may interpret the findings and correlate them to the clinical picture. Only at this stage, can we begin to have more effective treatments and rehabilitation protocols focused on breaking the pain cycle, improving mobility, and strengthening the cervical spine to support the horse through training. All of these steps must be taken in order to get the horses back to a state of well-being and be able to maintain that over time and through the rigors of their job or athletic endeavours.

Video 1

Video 1 highlights a horse that presented for suspect cervical pain and is hyperesthetic along the neck region. The horse is guarded and the thoracolumbar epaxial musculature is hypertonic, though the horse does not openly show pain upon myofascial palpation in that region. The horse does show pain to palpation along the middle gluteal muscle to the caudal sacrum. Mobilization of the cervical spine was not clinically concerning as the horse had moderate ability to laterally bend in both directions, however the horse shows severe aversion to mobilization of the thoracolumbar region. The horse had minimal abnormalities in the cervical spine on radiographs showing only mild enlargement at C6-C7. Ultrasonography showed mild osseous irregularity at C6-C7. At post-mortem examination, the gross findings included L5-L6 body ankylosis and L6-S1 dorsal disc protrusion with dural petechial hemorrhage. Histopathology showed severe ganglionitis at C4-C6 and lymphocytic ganglionitis and neuronal loss from T10-T18 and lumbosacral perineural hemorrhage. These complex findings highlight the importance of recognizing that while a horse may show cervical region hyperesthesia, that may in fact not be the primary or only significant region of interest.

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CHAPTER 2:
DANGEROUS BEHAVIOR AND INTRACTABLE AXIAL SKELETAL PAIN IN PERFORMANCE HORSES: A POSSIBLE ROLE FOR GANGLIONEURITIS (14 CASES; 2014-2019)[2]

Introduction

It is all too common that a rider purchases a new horse with excitement and high aspirations, but because of health or training issues, those expectations are never realized. These training limitations sometimes progress to dangerous behavior such as kicking out, refusing to go forward, bucking and rearing. The horse is then characterized as a problem horse, and training methods may become more punitive. Undesired behavior in horses most often stems from their attempt to avoid fear or pain (1, 2). Horses may develop undesirable traits that progress to dangerous behavior for a multitude of reasons that include lack of clear communications or use of aids, improper training, lameness (3), poor saddle fit (4), axial skeletal pain (5), and gastrointestinal or reproductive abnormalities (6). Some trainers acknowledge that the bad behavior may stem from undiscovered physical problems and they enlist the help of medical professionals. Routine therapies are often applied, and the horse is asked to go back to work; however, the behavioral concerns continue or are only alleviated for short periods of time. A therapeutic trial of non-steroidal anti-inflammatory (NSAIDs) medication over several days followed by ridden exercise can be used to determine whether bad behavior may have an underlying inflammatory or pain component (7). Although a negative response (i.e., no improvement to NSAIDS) does not preclude the presence of pain (8), this response may reinforce the perception that the affected horse has behavioral issues and needs more aggressive

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training. Unfortunately, this approach may exacerbate the underlying pain behavior. The horse may become more dangerous until the owner or trainer eventually give up and sell the horse; only for the process to be repeated with a new trainer and veterinarian. After much expense, and long durations of frustrating diagnostics and trials of ineffective treatments, owners may finally opt to euthanize the horse; for the safety of the rider and the well-being of the horse. If these horses are euthanized, a routine necropsy (9) often fails to provide any additional insights as to the cause of the dangerous behavior. The owners are eventually left wondering whether euthanasia of their horse was justified due to the lack of clinically significant pathologic findings.

Over a period of 5 years, the authors have identified a group of young to middle aged performance horses that became difficult to train and ultimately dangerous to ride within a short time after purchase. These horses were often very well-behaved and easy to handle for general care. However, when asked to work under tack or advance in training, they showed dangerous behavior such as bucking, refusing to go forward, and rearing to the extent that they were too dangerous to ride. After repeated and extensive musculoskeletal evaluations which included diagnostic anesthesia, diagnostic imaging, and the lack of response to numerous applied therapies, these horses were euthanized due to their dangerous behavior. This paper reports a series of 14 affected sports horses (representing 0.001% of horses presenting for evaluation in the same time frame), including performance history and previous lameness evaluations (retrospective component); and a detailed behavioral, physical, lameness and neurologic examination, diagnostic imaging, and gross and histologic examination of the vertebrae, spinal cord and dorsal root ganglia (prospective portion).

Materials and Methods

Case Selection

The Institutional Animal Care and Use Committee (IACUC) at Colorado State University (protocol number 1371) reviewed and approved the study. Written informed consent was obtained from the owners for the participation of their animals in this study. Young to middle aged horses initially intended to be used for performance, who were euthanized due to severe behavioral or training issues judged to be too dangerous to themselves or the rider, were included in the study. The owners' chief complaint and description of the concerning behaviors were recorded. All available records from previous diagnostic evaluations and applied treatments were reviewed and recorded. All horses had prior veterinary examinations and multiple treatment regimens that failed or were only effective for a short period. The region of concern from the owner's or treating veterinarian's perspective was typically localized to the axial skeleton, with a strong suspicion that the cervical region was the primary source of the pain and subsequent dangerous behavior.

Spinal Examination

The spinal evaluation procedure included myofascial (i.e., soft tissue) palpation and vertebral mobilization and was performed by a dual-boarded equine surgeon and sports medicine and rehabilitation specialist certified in veterinary acupuncture and chiropractic (MS) (10). The myofascial examination was used to assess the behavioral responses to light touch, tone and texture of the superficial soft tissues, and development of the epaxial musculature (e.g., the semispinalis capitis, splenius, longissimus, iliocostalis, and middle gluteal muscles) (Video 2).

The skin was evaluated for the ability to glide freely over the underlying superficial fascia and muscles without any resistance or sensitivity. The soft tissues were palpated from superficial-to-deep, assessing signs of inflammation, such as heat and swelling, tissue texture, muscle tone, and areas of sensitivity within the different tissue layers (11). The quantity (e.g., affected tissues, area and depth) and the quality (e.g., severity) of any abnormal findings were recorded. The presence, location and severity of muscle asymmetry were also noted (12). The quantitative (e.g., range of motion) and qualitative (e.g., ease and fluidity) characteristics of both passive and active (i.e., baited carrot stretches) lateral bending of the cervical and thoracolumbar regions were recorded. Vertebral segment mobilization throughout the axial skeleton was assessed in lateral bending and flexion-extension (13). Compression of the tubera sacralia and bilateral ventral mobilization of the tubera coxae were performed to evaluate lumbosacral (LS) and sacroiliac (SI) joint motion and reactivity. Scapular motion was used to evaluate caudal cervical to cranial thoracic (C7-T4) mobility (cervical lateral bending and dorsal scapular motion) and pain (backing away, pulling the limb away, or rearing). The clinician induced dorsal scapular motion via passive elevation of the entire unweighted forelimb by holding the metacarpal region parallel to the ground while flexing the limb at the carpus. This was performed with concurrent ipsilateral passive lateral bending of the head and neck, to the extent the horse would allow, by an assistant. All parameters were graded as normal (no restriction), mild, moderate or severe restrictions within each spinal region.

Behavioral Responses

Behavioral responses noted during the myofascial and spinal mobility examinations were graded (by MS) as normal, mild, moderate, severe, or too dangerous to evaluate (14). Normal behavior

was characterized as no observable response to palpation or mobilization of any spinal region. Mild behavioral responses consisted of slight reactions to deep pressure, the presence of mild hypertonicity or stiffness (lack of normal range of motion of joints or flexibility of soft tissues), and a single spinal region affected. A moderate behavior score was assigned if the horse reacted to moderate pressure, the presence of moderate hypertonicity or stiffness, and if several spinal regions were affected. Moderate behavioral reactions included the horse holding the ears back behind vertical, attempts at biting or kicking, and moving away from the examiner. Severe behavioral reactions were noted in horses that were hyperreactive to any applied touch, would try to bite the handler, continuously move away from the examiner, and had a strong resistance to any induced spinal mobility within multiple vertebral regions. Horses categorized as too dangerous to evaluate displayed aggressive behaviors such as biting, kicking, striking and rearing, which prevented examiners from approaching or touching them.

Gait Evaluation

The lameness examination (performed by MS) included walking and trotting in hand on hard ground in a straight line and in 15 meter circles in hand in both directions and was graded 0 - 5 per the American Association of Equine Practitioners (AAEP) lameness scale (15). The neurologic evaluations were performed by YNL (dual-boarded in critical care and internal medicine with clinical emphasis on equine neurologic diseases). The neurologic examination included a cranial nerve examination, walking in a straight line with the head held in neutral and elevated positions, walking in small circles to evaluate forelimb and hindlimb placement, backing the horse up for several strides, and evaluating the response to lateral tail traction while standing still and during walking. Additional neurologic challenges including walking the horse

up and down a small incline and over a curb were included dependent on animal compliance and environmental circumstances. Ataxia was graded 0-5 based on the modified Mayhew scale (16). Dysmetria and weakness were graded as absent, mild, moderate, or severe.

Diagnostic Imaging

All previous imaging studies performed at the Colorado State University Veterinary Teaching Hospital had been evaluated by board certified radiologists on clinical duty (MB, KS) and these records were evaluated and recorded. At the time of enrollment, an updated diagnostic imaging evaluation (radiographs, ultrasound, ex-vivo computed tomography [CT]) of the cervical region was completed. Cervical radiographic evaluation (Toshiba 1700) was performed by a blinded observer (MB), and included lateral-lateral radiographs of the occiput to the first thoracic (T1) vertebra. Oblique projections were also included as indicated to clarify abnormalities noted on the lateral cervical radiographs. Radiographic images were evaluated for enlargement of the APJ, (e.g., osteophyte and enthesophyte formation), and the size of the intervertebral foramina. Radiographic findings were graded as normal, mild, moderate or severe (17). Ultrasound examination (GE Healthcare, Logiq 90, 12 MHz linear probe, or the Toshiba Aplio i700, 10MHz linear probe) of the cervical region was performed bilaterally from the occiput to T1 vertebrae (18). Ultrasonographic images were evaluated in real-time during image acquisition and consensus graded (MB, MS). The cervical APJ margins and joint capsule enthesis were graded as normal (no abnormalities), mild (small area of bone proliferation or irregular surface), moderate (moderate bone proliferation and irregular surface), and severely affected (substantial bone proliferation or fragmentation noted). The intervertebral foramina and presence or absence of joint effusion were also evaluated and graded as normal or abnormal. Joint capsule thickness

was subjectively evaluated as normal or thickened. CT (Gemini Big Bore, Philips Healthcare, Andover, MA) was used for ex vivo evaluation of the cervicothoracic region (C1 to T5). CT images were consensus graded by MS and a blinded, board-certified equine radiologist (KS) (19). The size of the APJs were graded as normal, mild, moderately or severely enlarged. The medial and lateral articular margins and the joint capsule were evaluated for the presence of osteophytes and enthesophyte, respectively. All regions were graded as normal, mild, moderate, or severely affected. The subchondral bone of the APJ was scored as either normal or irregular. The cervical intervertebral foramina were evaluated and graded as normal or narrowed, using the cranial and caudal foramina to compare size. The nerve roots were graded as normal or enlarged. The width of the intervertebral disc (IVD) space was graded as normal or narrowed, and the dorsal profile of the IVD was graded as normal (no protrusion), mild, moderate, or severely protruding.

Gross Pathologic Examination

Horses were euthanized by intravenous administration of barbiturates (Pentobarbital 390 mg/ml, 60 ml) and immediately transported to the necropsy facility. The head and cervical region were collected en bloc via transection at the T5-T6 vertebral level and immediately transported for CT imaging. Following CT imaging, the soft tissues were removed from the cervicothoracic specimen, which was sectioned through the vertebral bodies within the frontal plane to allow en bloc removal and histologic processing of the spinal cord, nerve roots and dorsal root ganglia (DRG). Gross examination of the neural tissues included evaluation of the brain, spinal cord, venous plexus, and DRG (C1-T3) for alterations in color and presence of induration and/or nodularity. The DRG were considered abnormal when enlarged, firm and nodular. Attempts

were made to routinely collect bilateral DRG from all cervical vertebral levels in all horses. When indicated based on clinical or lameness examinations, the ipsilateral brachial plexus (only the proximal brachial plexus, not the nerves contributing to the plexus) and the thoracolumbar, lumbosacral or sacropelvic regions were also collected and evaluated grossly for soft tissue, osseous and neural pathology. Gross examination of the osseous and articular structures was performed by a blinded observer (KH) who was aware of the general regions of concern within each horse, but who had no specific knowledge of the clinical or diagnostic imaging findings. The vertebral bodies were examined for the presence of spondylophytes and the IVD were examined grossly for signs of disc degeneration, which was subjectively graded as normal (no degeneration), mild, moderate and severe using a prior grading system (20). The joint capsules of the APJs were resected to allow complete visualization of the articular surfaces and joint margins for signs of articular cartilage damage, osteophyte and enthesophyte production, and modeling of the articular surfaces and joint margins (21). Articular changes were graded as normal, mild, moderate or severely affected using previously reported scoring methods (21). The amount of joint capsule thickening and presence of synovial fold hyperemia was also noted.

Histopathology

The DRGs from the cervical, thoracolumbar, and lumbosacral regions were collected and processed for histologic evaluation. The DRG were bisected with one-half fixed in 10% neutral buffered formalin (NBF) and the other half fresh frozen (-80°F for future evaluation). Transverse and sagittal sections of dorsal nerves and ganglia were processed and embedded in paraffin. Sections were cut (5-6µm) and attached to egg albumin-coated slides and then were stained using routine hematoxylin and eosin (H &E), Masson's trichrome, and Luxol's fast blue using

the respective procedures established at the Colorado State University, Veterinary Diagnostic Laboratories. Sensory nerve rootlets and their corresponding DRG were histologically evaluated for cellularity, fibrosis, the formation of lymphocytic nodules or hyperplasia of satellite cells, and the presence of Nageotte nodules. A diagnosis of spinal neuritis (neuroinflammation of the nerve roots), ganglionitis or both was based on gross and histological evidence of pathological alterations. Evidence of ganglionitis was histologically established if there was: significant hypercellularity due to infiltration by increased numbers of lymphocytes (perivascular cuffs or endoneurial infiltrates), macrophages including clustering of hemosiderin-laden histiocytes, hyperplasia of satellite cells, fibrosis and drop-out of ganglionic neurons (22). Fibrosis was further confirmed through staining with Masson's trichrome. Drop-out of ganglionic neurons was evident as many neurons were almost totally obliterated by neuronphagia. DRG from non-affected regions (i.e., most commonly C1-C2) served as intra-horse histologic controls. DRG from 3 clinically (riding-age horses, euthanized for owner-related reasons, not showing any signs of allodynia or hyperalgesia or having a history of axial skeleton concerns) and histologically normal horses served as histologic references (unpublished data; TA and KH). Control ganglia from each corresponding horse or clinically normal (no pain on clinical examination) control horses had a total score of < 400 nucleated cells (excluding neuronal and fibroblasts). Mildly affected ganglia had increased cellularity >400<500 cell/ high power microscopic field (HPMF); moderate ganglionitis >500<600 cell/HPMF; and severe ganglionitis >600 cell/HPMF. Based on clinical examination findings, the proximal brachial plexus (the individual nerves were not evaluated separately) in select cases was evaluated. The brachial plexus was considered normal if the bundles of nerves were merged together and ensheathed with perineurium with a regular contour. There was no nodular infiltrate in the endoneurium or epineurium. As has been

described in laboratory animals (23), signs of inflammation of the plexus included perineural fibrosis and multifocal perivascular cuffs comprising moderate numbers of lymphocytes, plasma cells and hemosiderin-laden macrophages.

Clinical Case Summaries

An aggregate assessment was used to determine the primary spinal region of concern. The aggregate was formed using all available information which included the medical history, spinal examination and behavioral response, gait evaluation, diagnostic imaging results, and gross and histopathologic evaluations.

Results

Horses and Owner Complaints

Fourteen horses that developed severe performance limitations and dangerous behaviors under saddle that eventually resulted in euthanasia were included in this case series. The mean age was 9.4 ± 2.6 years and included eleven geldings and three mares. The breeds included eight warmbloods, four Thoroughbreds, one Quarter Horse, and one Andalusian. Eight of the horses were used for dressage, four for eventing, one for show hunting, and one for barrel racing. The owners' chief complaint and reports of dangerous behavior were recorded (Table 1). In 12 horses, the time from purchase to euthanasia was 2.5 ± 1.8 years. The other two horses were raised by their owner, and the time from initial complaint to euthanasia was less well defined. Ten (71%) owners' primary complaint was of dangerous behavior. Professional riders rode the remaining four horses and the primary complaint was performance limitations; two riders

complained of aggressive behavior, one falling down and one for bruxism and inability to back up.

Spinal Examination and Behavioral Responses

Eleven (79%) horses were extremely reactive to initial palpation and light touch of the skin and superficial fascia (Table 2). Ten (71%) horses exhibited severe behavioral responses throughout the myofascial and mobilization examinations, which included kicking, biting, moving away, striking or rearing, which made physical examination difficult and dangerous (Video 3-5). One horse displayed severe avoidance and dangerous behavior (i.e., too dangerous to evaluate) that precluded any additional palpation or mobilization examination. The remaining three (21%) horses displayed moderate behavioral responses (e.g., bracing to palpation or subtle increased tension in the muscle, ears back, tension in the eyes). All horses which were palpated (13; one horse was unable to be palpated due to the dangerous behavior) showed some degree of abnormal reactivity to palpation of the left and/or right brachiocephalicus muscle(s) (Table 2). Five (38%) horses displayed moderate to severe signs of reactivity to palpation and mobilization of the withers and scapular regions. Ten (77%) horses had moderate to severe abnormalities localized to the thoracolumbar region, which included hypertonic epaxial muscles and resistance to mobilization in lateral bending or flexion and extension. Seven (54%) horses had moderate to severe adverse reactions to axial compression of the tubera sacralia, ventral mobilization of each tuber coxa, or stimulated pelvic flexion. Five (38%) horses had reactivity to palpation at the proximal attachment of the semitendinosus muscle (unilateral or bilateral, Table 2) along the lateral aspect of the sacrum (S3-S5).

Gait Evaluation

Thirteen (93%) horses displayed lameness in at least one fore or hind limb. No horse had greater than grade 3 lameness in any limb (subtle, but present, lameness noted in a straight line). Seven horses displayed bilateral forelimb lameness, three had unilateral forelimb lameness, three horses had a unilateral hind limb lameness, and four horses had both fore and hind limb lameness. A complete neurologic examination was completed in 13 of the 14 horses (one horse was not available for a neurologic examination before euthanasia). None of the horses displayed signs of pelvic limb ataxia. One horse displayed bilateral thoracic limb hypometria when walked, and when walked with a raised head this dysmetria worsened. No pelvic limb gait abnormalities were noted in this horse. One horse showed mild thoracic limb and moderate pelvic limb weakness, and one horse showed signs of mild hypermetria in both pelvic limbs.

Diagnostic Imaging

Diagnostic imaging modalities used over the year(s) prior to enrollment into the study included radiography, ultrasonography (percutaneous and transrectal), and nuclear scintigraphy. These imaging modalities were utilized during lameness evaluations before acceptance into the study and results were available for ten horses (Table 3). Prior diagnostic imaging included cervical (N=8) and thoracolumbar radiographs (N=5). Ultrasonographic examinations of the axial skeleton included cervical (N=4), thoracolumbar (N=5) and transrectal (N=8) evaluations. Mild (N=4) or moderate (N=2) periarticular bone proliferation of the APJs, at various levels from T16-L6, was noted in all horses with thoracolumbar ultrasound examination. Transrectal ultrasound examination (24, 25) revealed two horses with periarticular bone proliferation of the right SI, and one horse had bilateral SI proliferation. Five horses had abnormalities noted at the

LS junction which included narrowing of the LS disc space, fibrosis or mineralization of the LS disc, or L6 endplate remodeling. Due to poorly localized musculoskeletal pain or dysfunction, six horses underwent full body skeletal scintigraphy examination. On nuclear scintigraphy, one horse had mild, diffuse increased radiopharmaceutical uptake in the region of the C4-C5 APJ, and one had moderate, diffuse increased radiopharmaceutical uptake in the region of the C6-C7 APJ (26). Five horses had increased radiopharmaceutical uptake in the thoracolumbar spinous processes (N=4) or the APJ (N=3), and three horses had increased radiopharmaceutical uptake in the sacrum and/or ilium close to the right SI joint.

At the time when owners elected euthanasia, the horses were enrolled in the study. All horses had updated diagnostic imaging of the cervical spine, including radiographic, ultrasonographic, and ex-vivo CT imaging (Table 3). On radiography, eight horses had mild (N=5) or moderately (N=3) enlarged cervical APJs and one horse had narrowed C6-C7 intervertebral disc space. Ultrasonographic evaluation revealed APJ periarticular bone proliferation; mild (N=5), moderate (N=5) and severe (N=1) at multiple levels between C2-T1. CT examination of the cervical region revealed enlargement of the APJs (N=6), periarticular proliferation (N=12) of the APJ, and IVD protrusion at multiple levels between C2-T2: mild (N=6), moderate (N=6) and severe (N=1). Lobular, hyperattenuating regions on CT examination were interpreted as epidural hemorrhage at C1-C2 (N=1) and T4 (N=2), and subarachnoid hemorrhage T4-T5 (N=1).

Gross Pathologic Examination

The initial horse enrolled into the study lacked a detailed gross examination of the cervical vertebral column. Within all subsequent enrolled horses (N=13), moderate to severe osteophytes of the cervical APJ (N=11) and moderate to severe intervertebral disc disease (N=7) localized to the caudal cervical region (Table 4) were evident. Dural hemorrhage at C7-T1was noted in 2 horses. In horses that the thoracic vertebral column was grossly evaluated (N=8), dural hemorrhage within the cranial thoracic region (N=2), and moderate intervertebral disc degeneration (N=3), were noted. Impingement of the spinous processes at T11-T17 was noted in two horses, with moderate APJ modelling at the same vertebral levels of the impingement. The lumbar vertebral column was evaluated in ten horses with intertransverse joint ankylosis noted at L4-L5 (N=1) and L5-L6 (N=4), and moderate to severe L4-L6 APJ modelling (N=4). Narrowed intervertebral disc space and or disc protrusion was noted from L5-S1 (N=4) with dural hemorrhage present at L4-L6 in one horse. The sacrum and pelvis were evaluated grossly in twelve horses, with moderate to severe SI joint modelling (N=7) and lumbar sacralization (N=3) noted. Dural hemorrhage at the lumbosacral junction was seen in four horses.

Histopathologic Examination

All horses in this case series (N=14) had multiple levels of moderate to severe ganglioneuritis (Table 5). In contrast, the DRG in control horses (N=3) had normal cell counts and lacked evidence of any pathologic changes. In affected DRG, the ganglionic neurons (perikarya) had partial to total clearing of perinuclear cytoplasm (chromatolysis). Within the moderately and markedly inflamed ganglia, drop-out of neurons and replacement with fibrosis was evident. In the most affected neurons, nuclei were shrunken and hyperchromatic (pyknotic). Surrounding

scalloped perikarya were several layers of hyperplastic satellite cells, which encroached upon degenerate or necrotic perikarya forming >3 Nageotte bodies per a 400x HPMF (Figure 1). Histologic evidence of brachial plexitis (i.e., inflammation of the brachial plexus; N=4) and acute hemorrhage (epidural to subdural; N=9) was noted. No other clinically significant findings were noted within the central nervous system during the post-mortem examination.

Clinical Case Summaries

The aggregate assessment of the most commonly affected vertebral region included the cervicothoracic region (N=7), lumbosacral region (N=5), cervical (N=1) and an aggregate assessment could not be made in one horse because the dangerous behavior disallowed palpation (Table 6). Moderate to severe ganglionitis was noted within the aggregate region of interest (N=12). Based on gross post-mortem examination, the lumbosacral region was the most severely affected region. Across all horses, the cervical and cervicothoracic regions were judged to be the most commonly affected sites (Table 6). The thoracolumbar region was not considered the primary region of concern within any horse.

Discussion

This case series suggests that the dangerous behavior noted in these horses was more likely due to the presence of neuropathic (i.e., structural) pain rather than due to "bad" behavior or poor training. We hypothesized that physically identifiable lesions would be found within the cervical region at post-mortem examination (i.e., gross and histopathologic evaluation), that would explain the observed adverse behaviors despite extensive, yet inconclusive, prior diagnostic imaging, and the lack of response to numerous applied treatments. In preliminary work, routine

gross and histologic evaluation of the central nervous system in six horses (not included in this series) failed to reveal any clinically significant pathologic findings within the brain or serial sections of the spinal cord; therefore, we began to expand our search to the DRG, spinal nerve roots, and nerves. Ganglionitis alone or ganglioneuritis was diagnosed in all horses within this case series; however, the DRG lesions were not specifically localized to the cervical region. As the clinical study progressed, it became apparent, based on painful responses to myofascial and mobility examinations throughout the entire axial skeleton, that all spinal regions should be thoroughly evaluated at necropsy. In research models, it has been shown that the DRG may be affected several spinal segments from a site of structural pathology (27). Therefore, we expanded our search to include the entire axial spine in order to capture gross and histologic information that would have been overlooked if our investigation was limited only to the cervical region.

The DRGs contain the cell bodies of afferent sensory neurons and serve an important role in relaying peripheral sensory information to the central nervous system (28). DRG are located within, or close to, the intervertebral foramina, the size of which may be compromised by changes in posture or the presence of adjacent soft tissue or osseous proliferation (e.g., osteophytes or disc protrusion). This may then lead to DRG injury which could cause the neurons to become hyperexcitable, which in turn might result in spontaneous firing (28). Satellite glial cells (SGC) are found wrapped around the neuronal cell bodies in the DRG where they play a role in neuronal homeostasis. When there is nerve damage or inflammation, the SGC become activated, resulting in neuronal hyperexcitability and consequent pain (29). The vasculature of the DRG is permeable, unlike the blood-brain barrier present within the central nervous system (30). This lack of barrier allows local inflammatory mediators (i.e., secondary to IVDD or APJ

OA) direct access to the DRG and subsequent activation of the SGC (29-31). These cellular and molecular responses at the level of the DRG, secondary to inflammation or nerve damage, facilitate chronic pain (32).

In humans, ganglionitis has been documented in chronic, neuropathic pain syndromes (32, 33). Similarly, ganglionitis has been reported in chronic, pathologic pain syndromes in horses associated with laminitis and idiopathic forelimb lameness (34-38). In this case series, all horses had moderate to severe ganglionitis identified at multiple vertebral levels which we theorize to be related to the observed dangerous behavior and apparent neuropathic pain. However, a thorough evaluation of the cellular and molecular markers from DRG acquired from a normal sports horse population would greatly improve our interpretation of the clinical relevance of these histopathologic findings.

The cervicothoracic region (C7-T4) was the most common region identified as the site of pain and dysfunction, followed by the lumbosacral junction (L6-S1) and then cervical region (C2-C7). The neuroanatomical localization of neuropathic pain to the cervicothoracic region (C7-T4) and brachial plexus was based on the presence of forelimb lameness and ipsilateral reactivity to dorsal scapular mobilization combined with ipsilateral bending of the neck. Interestingly, brachial plexus injuries are highly associated with the presence of neuropathic pain in humans and include inflammatory brachial plexopathies and plexitis due to idiopathic, traumatic (39), viral, bacterial and immune-mediated mechanisms (40). Similar inflammatory and immunologic mechanisms need to be explored in horses.

A common clinical finding in this case series (71%) was identifying subdural and epidural hemorrhage and/or hematomas, that were most commonly present at the cervicothoracic and lumbosacral junctions. In humans and dogs, epidural and subdural hematomas occur (41-44), and are considered surgical emergencies (45). In contrast to this case series, pain associated with spinal hematomas in humans and dogs is usually acute, intense, and generally associated with neurologic deficits caused by spinal cord compression (42, 46). In similar fashion to our report, spontaneous epidural hematomas in humans occur more commonly in the high mobility areas of the cervicothoracic and thoracolumbar spine (47). Epidural hematomas localized to the cervicothoracic region have been reported in the equine literature. However, the horses in that report all displayed ataxia attributed to spinal cord compression associated with the hematoma (48). In that series, Gold et al. reported hematomas to be chronic in nature, as fibrin and hemosiderin-laden macrophages were evident in those lesions. The etiology and pathogenesis of spinal hematomas in human medicine is difficult to define as many different classifications have been used such as; spontaneous, idiopathic, traumatic, coagulopathic, and other (42). Similar mechanisms that may affect vascular fragility within the vertebral canal need to be explored in horses.

Pain is defined as “an unpleasant sensory and emotional experience” (49) and is categorized as nociceptive, inflammatory or pathological (50). Nociceptive pain is protective and serves to limit contact with noxious stimuli through the withdrawal reflex. Inflammatory pain, often as a result of injury or surgical intervention, is also protective and commonly managed with the administration of NSAIDs. Pathological pain is not protective and can be divided into neuropathic pain (i.e., structural neural lesion) or dysfunctional pain (i.e., neuropathologic

functional disorder) (50, 51). Pathologic pain, without an inflammatory component, is unlikely to respond to NSAIDs. Hyperalgesia, defined by the International Association for the Study of Pain (IASP), is “increased pain from a stimulus that normally provokes pain”, and allodynia is pain caused by stimulation that does not typically cause pain (52), both are frequently associated with neuropathic pain (53). Neuropathic pain in humans can be spontaneous, and does not need to be associated with ongoing tissue damage (54). This has been described in horses (37, 55) and may also be true in this series.

Given that most horses accept human touch, 71% of horses is in this report were judged to be allodynic. As has been proposed in human medicine (53), signs of allodynia and hyperalgesia may be useful indicators of the presence of neuropathic pain in horses (56). In addition to the importance of clinical examination findings, pain questionnaires in human medicine have also been shown to be helpful in the diagnosis of neuropathic pain (57). In equine practice, it is also critically important to listen to the rider or owner describe their horse’s behavior to increase awareness of subtle pain behaviors (58, 59). In this series, the owner and trainer’s initial complaint and historical account were very similar in nature. There was a common thread of recent purchase (i.e., within three years) with the intent for each horse to be used for athletic endeavors, but never able to achieve the level of intended use due to the development of dangerous behavior. There was also a failure to resolve dangerous training issues after multiple diagnostic and therapeutic attempts, which together should alert veterinarians to the possibility of severe underlying disease processes and the potential development of associated neuropathic pain. The authors believe this lack of response to routine therapies (e.g., NSAIDs, intra-articular corticosteroid treatments) is an important indicator that a horse may be experiencing neuropathic

pain. In contrast, dangerous behavior that is readily modified with diagnostic analgesia is much more likely to be due to inflammatory pain (3).

Most diagnostic imaging modalities are limited to providing a pathoanatomic diagnosis; however, a collection of medical history and clinical examinations, in addition to diagnostic imaging findings, may help to identify underlying disease mechanisms that may contribute to the development of neuropathic pain. In the cervicothoracic region, vertebral endplate sclerosis and narrowing of the intervertebral disc as seen on radiographs, or protrusion of the dorsal intervertebral disc identified on CT support the diagnosis of chronic vertebral instability and IVDD (55). In humans, IVDD has been associated with the development of neuropathic pain (60). The radiographic diagnosis of impinged thoracolumbar spinous processes may be of little clinical significance in some horses (61, 62), but is likely an important radiographic finding in horses that have notable pain responses to digital palpation along the dorsal midline, epaxial muscle hypertonicity or atrophy, and severe avoidance behavioral responses to spinal mobilization (e.g., flexion-extension) of the affected spinal region. The ventral aspect of the lumbosacral region is frequently evaluated with transrectal ultrasonography; (63) however, varying degrees of irregular intertransverse or sacroiliac joint margins can be noted in horses that do not have obvious clinical signs of pain or dysfunction localized to this region. More recently, changes in intervertebral disc echogenicity at L5-L6 and the lumbosacral intervertebral levels have been associated with regional pain and poor performance (64). Similarly, horses in our study with reactivity localized to the lumbosacral region often had evidence of L6 sacralization, ankylosis of the lumbar intertransverse joints, or lumbar and sacroiliac osteoarthritis. These horses would frequently buck or kick during manual palpation of the lumbar epaxial muscles,

consistently unlock both stifles during tubera sacralia compression, and have severe adverse reactions to ventral mobilization of the tubera coxae.

When presented with complicated cases such as these, an aggregate assessment, which incorporated the primary findings across all outcome parameters and owner complaints, proved useful in defining a specifically affected spinal region. In this case series, the histopathologic evaluation was considered the gold standard for a definitive diagnosis and localization of the neurologic lesions. As hypothesized, organic lesions of the nervous system were identified, however there was no single antemortem modality or examination finding that clearly indicated the exact site of the pathoanatomic lesions in this case series. While it is likely that there is a causal relationship between the clinical features and post mortem findings, this is difficult to determine without more control horses and grading of lesion severity.

Limitations

A limitation of this study is the lack of complete data sets within each horse. As a case series, there was clinical variation in the available retrospective data available. Additionally, inadvertent oversight or the inability to collect gross and histologic tissue samples at all vertebral levels and the brachioplexus prevented complete analysis in some horses. We used three control horses to provide comparisons; however, the incorporation from additional unaffected (non-painful) age-matched sports horses would have helped to expand our understanding of the relationship between ganglionitis and pain-behavior.

Conclusion

The purpose of this case series is to raise awareness and acknowledge that severe behavioral problems in horses may be due to lesions of the nervous system resulting in neuropathic pain. This case series highlights the need for a more in-depth understanding of pain behavior and its clinical presentation and progression in severely affected horses that do not respond to traditional therapies used to treat musculoskeletal pain or lameness. The client and trainer perspectives are critically important to recognizing pain behavior. The myofascial and spinal mobility examinations provided critical information to identify clinical signs that justified the horse's unwanted or dangerous behavior, and helped to localize the spinal regions of interest. When this localization has been established, advanced diagnostic imaging modalities may be instituted, focusing on the highly mobile cervicothoracic, thoracolumbar and lumbosacral regions. The overall objective is to develop an early diagnosis and effective treatment of neuropathic pain syndromes in horses so that they may live full, productive, and pain-free lives.

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

Author Contributions

MS, TA, KH, YNL, WM contributed to study design and review of the manuscript. MS contributed to the acquisition of cases, review of records, myofascial and lameness examinations, review of diagnostic imaging, and manuscript preparation. KH, TA performed the post-mortem

examinations (gross and histopathology). YNL performed neurologic examinations. MB, KS provided diagnostic imaging analysis. All authors gave final approval of the manuscript.

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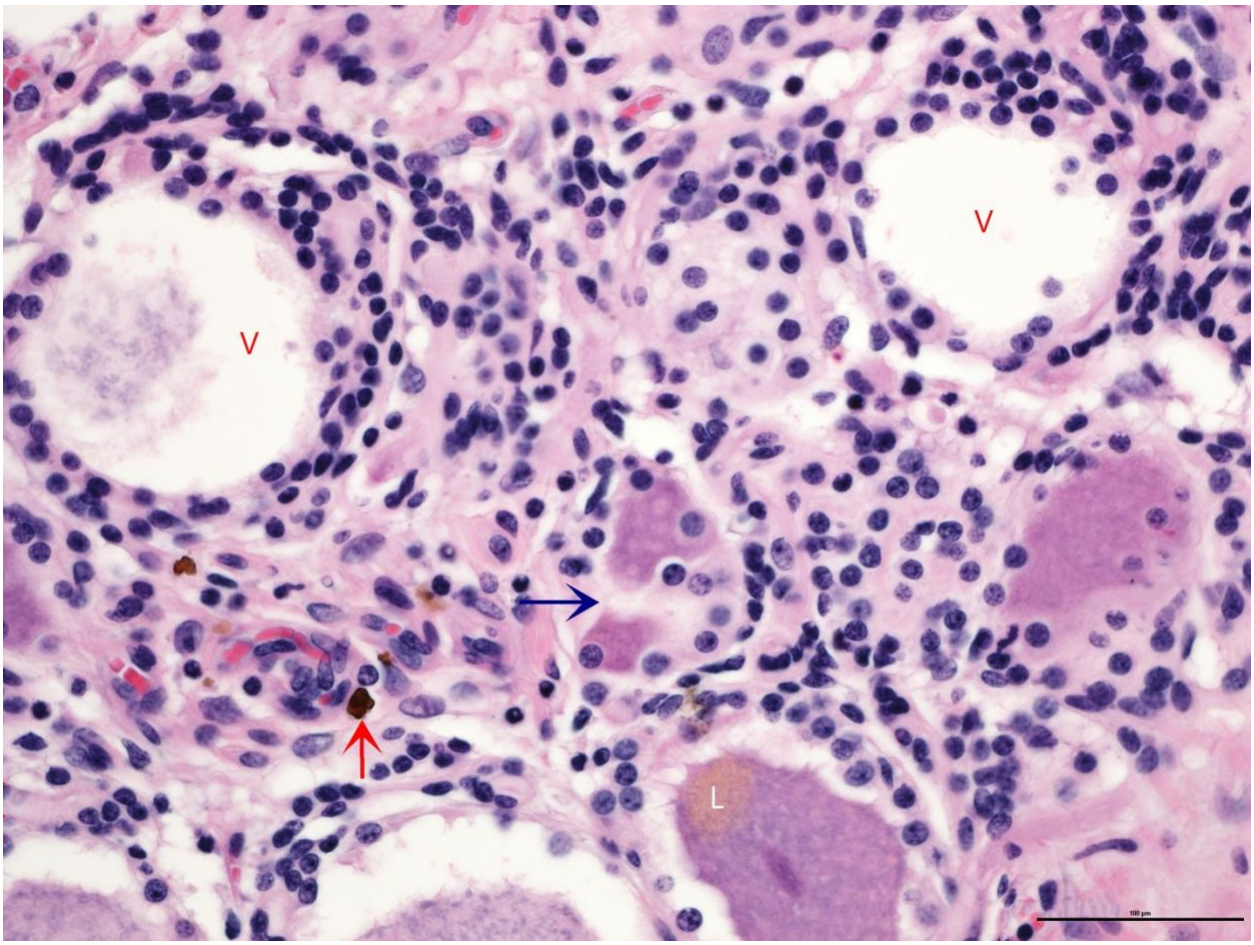

Figure 1. Transverse section of a dorsal root ganglion showing neuronal cell bodies and their corresponding axons. Partial to complete absence of cytoplasm is evident in the larger vacuolated neurons (V), which are surrounded by increased numbers of satellite cells that appear to encroach upon the periphery of perikarya engulfing degenerate soma along with fewer microglia (blue arrow). The lower neuron shows polar accumulation of brown pigment granules, lipofuscin (L), an incidental finding. Vessels are cuffed by small numbers of hemosiderin-laden macrophages (red arrow). Overall cellularity of the ganglionic stroma is significantly increased (N=723) (H and E, 400x).

Table 1. Presenting owner complaint and the interval of time from purchase to euthanasia. *Owner initially complained of training difficulties as a yearling. **Owned for 1.5 years prior to the initial presentation with a complaint of resistance to work and rearing. NA = Not available because these horses were raised from foals by the respective owners and underwent multiple periods of training and rest due to ongoing concerns.

Horse	Age at euthanasia (years)	Complaint	Owner Concerns	Interval (in years)
1	8	Performance limitations	Bruxism under saddle Shivers-like behavior, not able to back up Unable to advance in training	7*
2	9	Dangerous	Resistant to go forward Stumbling on the front limbs Rearing under saddle	1
3	7	Dangerous	Dangerous behavior under saddle	2
4	12	Dangerous	Violent, dangerous behavior under saddle	2.5
5	12	Dangerous	Resistance under saddle, difficult to collect Some rearing, progressed to falling down Suspected seizure activity	5**
6	9	Dangerous	Unable to lower head, not able to get into a dressage frame Violently throws head and panics while being ridden Nearly fell on the owner during a painful episode Bucked the owner off a twice	3
7	10	Dangerous	Bucking and explosive bolting under saddle	2
8	6	Dangerous	Resistance under saddle, won't move forward Bucking and rearing	NA
9	12	Performance limitations	Aggressively tossing head Falling down and collapsing which caused secondary trauma and multiple wounds	1.5
10	6	Dangerous	Intermittently unable to lower his head to the ground Leaping around, spooky and unrideable	2
11	14	Performance limitations	Hypersensitive Difficult to canter	1
12	11	Dangerous	Pinning ears, seems uncomfortable Progressed to dangerous behavior, unable to ride	1
13	10	Dangerous	Unpredictable and very nervous from the right side Unable to advance in training Trainer refused to ride	NA
14	6	Performance limitations	Reluctant to move forward especially at the canter Aggressive behavior	1.5

Table 2. Grade of behavioral responses, response to light touch, and positive findings and response to palpation and joint mobilization within spinal regions. BL = Bilateral, LB = Lateral bending, FE = Flexion-extension, C = Cervical vertebrae, T = Thoracic vertebrae, L = Lumbar vertebrae, TL = Thoracolumbar junction (T18-L1), LS = Lumbosacral joint (L6-S1), TC = Tubera coxae, TS = Tubera sacralia, NSF = No significant findings, NE = Not examined for safety reasons.

				Spinal Regions	
Horse	**Behavior**	**Touch**	**Cervical**	**Thoracolumbar**	**Lumbosacral and Sacropelvic**
1	Severe	Severe	Moderate pain BL brachiocephalicus m. Mild stiffness BL LB	Moderate hypertonicity BL epaxial m. Severe pain FE T10-L6	NSF
2	Severe	Severe	Moderate pain BL brachiocephalicus m. Mild stiffness left LB Moderate stiffness right LB	Moderate stiffness right T16-T17	Moderate focal pain right S3-S5
3	Severe	Severe	Moderate pain left brachiocephalicus m. Moderate stiffness right LB	Moderate stiffness right L3-L6 Severe pain right L3-L6	Moderate focal pain BL S3-S5 Moderate pain right SI joint
4	Severe	Severe	Moderate pain BL brachiocephalicus m. Moderate stiffness BL C4-C6	NSF	NSF
5	Severe	No	Moderate pain BL brachiocephalicus m. Moderate pain right poll region Severe pain left scapular elevation	Severe pain left T8-T12 Severe hypertonicity left T10-L6 Moderate pain BL L1-L6	Moderate focal pain BL S3-S5
6	Incapac-itated	Incapac-itated	NE	NE	NE
7	Moderate	No	Moderate pain BL brachiocephalicus m.	Moderate pain right T4-T12 Moderate stiffness right T8-T12	Moderate reaction TS compression
8	Moderate	Severe	Mild pain BL brachiocephalicus m. Moderate pain left splenius m.	Mild pain left T8-T12	Mild reaction LS flexion Mild focal pain BL S3-S5
9	Severe	Severe	Severe pain BL brachiocephalicus m. Mild stiffness BL LB	Moderate hypertonicity BL T10-L6 Severe reaction LB T12-T18	Moderate focal pain BL S3-S5 Moderate pain TC mobilization
10	Severe	Severe	Moderate pain BL brachiocephalicus m. Severe reaction right scapular elevation	Severe reaction LB T8-T12 Severe reaction extension T8-T16	Moderate reaction TC mobilization
11	Severe	Severe	Severe pain poll region Moderate pain BL brachiocephalicus m. Moderate stiffness BL LB	Moderate hypertonicity BL T10-L6 Moderate reaction BL T8-T12 Moderate stiffness T10-L6 Severe reaction extension TL	Moderate reaction LS extension
12	Severe	Severe	Moderate pain BL brachiocephalicus m. Moderate stiffness BL LB Moderate reaction left scapular elevation	Severe pain BL T8-T18 Severe reaction LB T8-T18 Severe reaction FE T8-T18	Severe pain LS flexion
13	Moderate	No	Moderate pain BL brachiocephalicus m.	NSF	NSF

			Moderate stiffness right LB		
14	Severe	Severe	Moderate pain BL brachiocephalicus m. Moderate stiffness BL LB	Moderate reaction T8-L6 Severe reaction right LB L3-L6	Moderate pain TS compression

Table 3. Pathologic findings localized to anatomical structures within spinal regions based on diagnostic imaging modalities. The positive findings of the listed modalities are color coded: nuclear scintigraphy (purple), radiography (blue), ultrasonography (black), and CT imaging (red). AP = Articular process joint, R = Right side, L = Left side, C = Cervical vertebrae, T = Thoracic vertebrae, L = Lumbar vertebrae, S = Sacral vertebrae, IVD = Intervertebral disc, IVF = Intervertebral foramen, SI = Sacroiliac, SP = Spinous process, NE = Not Examined, NSF = No significant findings, uptake = increased radiopharmaceutical uptake

		Spinal Regions	
Horse	**Cervical**	**Thoracolumbar**	**Lumbosacral and Sacropelvic**
1	NSF Mild enlarged AP C6-C7 Mild peri-articular bone proliferation AP C2-C4, C6-C7 Mild periarticular bone proliferation AP C2-T1 Mild non-articular irregular bone AP C2-C3 Moderate protrusion IVD C4-C5, C6-T1 Subchondral bone sclerosis (increased bone density) cranial AP C6-C7	Moderate uptake SP caudal T16-T18 Moderate uptake AP caudal T16-T18 Moderate impinged SP T18-L2 Mild periarticular bone proliferation AP T16-L1	Moderate uptake right SI joint Moderate periarticular bone proliferation right SI joint Mild periarticular bone proliferation left SI joint Moderate fibrosis IVD L6-S1 Mild endplate remodeling L6-S1
2	Mild uptake AP C4-C5 Mildly enlarged AP C6-C7 Mild non-articular irregular bone AP C2-C3 Narrowed IVD C6-C7 Mild lysis caudal endplate C6 Mild periarticular bone proliferation AP C3-C7 Mildly enlarged AP C3-C5, C6-C7 Mild to marked periarticular bone proliferation AP C3-C7 Mild non-articular irregular bone AP C2-C3 Mild irregular subchondral bone AP C2-C5 Moderate protrusion IVD C2-T1 Ventral narrowing and mineralization IVD C5-C7	Mild uptake AP T17-T18	NSF
3	Moderately enlarged AP C5-C7 Mild periarticular bone proliferation AP C4-T1 Mild enthesopathy AP C4-C6 Mild effusion AP C5-C7 Mild periarticular bone proliferation AP C4-C7 Mild enthesopathy AP C2-C5 Mild thickened joint capsule AP C5-C6 Mild protrusion IVD C6-T1	NE	NE
4	Mild periarticular bone proliferation AP C2-C7 Mild periarticular bone proliferation AP C3-C4, C6-C7 Moderate enthesopathy AP C2-C3 Moderate periarticular bone proliferation AP C3-C6 Moderate enthesopathy AP C2-C4 Moderate subchondral bone irregularity AP C4-C6	NE	NE

	Narrowed IVD C2-T1 Moderate protrusion IVD C4-C6 Narrowed IVF C2-C3 Enlarged nerve root C2-C3		
5	NSF NA NA	Mild sclerosis L3-L4 Moderate periarticular bone proliferation right L5-L6 Osseous irregularity IVF right L6	NSF
6	NSF	Mild increased uptake SP T16-T17	Mild diffuse uptake right SI joint
7	Moderate diffuse uptake AP C6-C7 Mild enlarged AP C6-C7 Mild periarticular bone proliferation AP C6-C7 Mild enthesopathy AP C4-C5 Moderate periarticular bone proliferation AP C3-C5 Mild joint capsule enthesopathy AP C2-C3 Thickened joint capsule AP C4-C5 Mild kyphosis C6-C7 Mild protrusion IVD C5-T1	Mild uptake SP T13-T18 Mild uptake AP L1-L2 SP impingement T13-T18 Mild periarticular bone proliferation AP L1-L2 Mild kyphosis T1-T4 Osteochondral fragment AP T2-T3 Epidural hemorrhage T4	Moderate diffuse uptake right SI joint Mild periarticular bone proliferation right SI joint
8	Mild enlarged AP C6-C7 Moderate periarticular bone proliferation AP C6-C7 Severe periarticular bone proliferation AP C6-C7 Moderate enthesopathy AP C6-C7 Moderate effusion AP C6-C7 Mild effusion AP C5-C6 Thickened joint capsule AP C5-C7 Mildly enlarged AP C6-C7 Mild subchondral defect AP C2-C4, C5-C6, C7-T1 Mild periarticular bone proliferation AP C2-C3, C4-C6, C7-T1 Mild protrusion IVD C4-C5, C6-C7 Thickened joint capsule AP C3-C4 Transposition of ventral tubercle C6 Mildly narrowed IVF C4-C5	Mild SP impingement T17-T18 Epidural hemorrhage T4	NE
9	Moderately enlarged AP C4-T1 Moderate periarticular bone proliferation AP C6-C7 Moderate periarticular bone proliferation AP C5-C6 Mild effusion AP C6-C7 Moderate thickened joint capsule AP C6-C7 Mild periarticular bone proliferation AP C7-T1 Mildly irregular subchondral bone AP C2-C4 Mildly thickened joint capsule AP C3-C4 Moderately thickened joint capsule AP C6-C7 Mild enthesopathy AP C4-C5 Severe protrusion IVD C6-C7	Mild periarticular bone proliferation AP T18-L1 Moderate periarticular bone proliferation AP L1-L2 Mild periarticular bone proliferation AP T1-T2	Mild periarticular bone proliferation BL SI joint Moderate mineralization IVD L6- S1 Mild bone proliferation IVF L6

	Mild protrusion IVD C4-C5 Slightly enlarged nerve roots C6-C7		
10	NSF Moderate periarticular bone proliferation AP C6-C7 Moderately enlarged AP C5-C6 Mild periarticular bone proliferation AP C3-C5, C6-C7 Mildly irregular subchondral bone AP C2-C6, C7-T1 Mild protrusion IVD C4-T1 Mild kyphosis and spondylosis C5-C6	Mild periarticular bone proliferation AP T1-T2 Mildly narrowed IVD T1-T4 Moderate protrusion IVD T1-T2 Subarachnoid hemorrhage T4-T5	NE
11	Mild enlarged AP C2-C3, C5-C7 Mild periarticular bone proliferation AP C2-C3, C5-C6 Moderate periarticular bone proliferation AP C2-C4 Mild periarticular bone proliferation AP C5-C7 Mild enthesopathy AP C2-C4 Mild effusion AP C6-C7 Mild periarticular bone proliferation AP C3-C4, C5-C6 Moderate protrusion IVD C3-C7 Epidural hemorrhage C1-C2 Moderate joint capsule enthesopathy AP C2-C3	Mild periarticular bone proliferation AP L3-L4 Mild periarticular bone proliferation AP T1-T2	Sacralization of L6
12	NSF NSF Mild periarticular bone proliferation AP C2-C3, C4-C5 Moderate periarticular bone proliferation AP C7-T1 Mild effusion AP C7-T1 Mildly enlarged AP C3-C5 Mild periarticular bone proliferation AP C3-C7 Mildly irregular subchondral bone AP C2-C4 Mild protrusion IVD C3-C7	Mild uptake SP mid to caudal T Mild SP impingement T17-L2 Mild periarticular bone proliferation AP T18-L3	NSF Narrowed IVD L6-S1 Sacralization vertebra L6-S1
13	Moderate enlarged AP C2-C3 Mild enlarged AP C5-T1 Mild periarticular bone proliferation AP C6-C7 Mild bone proliferation caudal occiput Mild periarticular bone proliferation AP C2-C5, C7-T1 Mild enthesopathy AP C3-C4 Moderate thickened joint capsule AP C5-C7 Moderately enlarged AP C2-C5 Severely enlarged AP C5-C7 Moderate periarticular proliferation AP C2-C7 Severe irregular subchondral bone AP C4-C7 Moderate protrusion IVD C5-C7 Mildly narrowed IVF C2-C3, C4-C7	NE	NE
14	NSF NSF Moderate periarticular bone proliferation AP C6-C7	NSF	NSF Severe narrowing IVD L6-S1 Sacralization of L6

	Mild periarticular bone proliferation AP C4-C6, C7-T1 Mild enthesopathy AP C6-C7 Mild effusion AP C6-T1 Mildly enlarged AP C5-T1 Mild periarticular bone proliferation AP C2-C7 Mildly irregular subchondral bone AP C2-C3, C5-C6 Mild protrusion IVD C3-C4, C7-T1 Moderate protrusion IVD C4-C5 Mild kyphosis C6-C7 Mildly narrowed IVF C6-T1		Moderately narrowed IVF S1 nerve roots

Table 4. Gross pathology findings within spinal regions. IVDD = Intervertebral disc degeneration, IT = Intertransverse, IVF = Intervertebral foramen, SP = Spinous process, AP = Articular process joint, BL = Bilateral, C = Cervical vertebrae, T = Thoracic vertebrae, L = Lumbar vertebrae, S = Sacral vertebrae, SI = Sacroiliac, NSF = No significant findings, NE = Not examined.

		Spinal Region	
Horse	**Cervical**	**Thoracolumbar**	**Lumbosacral and Sacropelvic**
1	NE	Ankylosis L5-L6	Narrowed vertebral canal L6-S1 Dorsal protrusion IVD L6-S1 Dural hemorrhage L6-S1
2	Severe periarticular bone proliferation AP C7-T1 Severe IVDD C4-T1 Dorsal protrusion IVD C6-T1 Dural hemorrhage C7-T1	Moderate spondylophyte T14-T16 Ankylosis L4-L5 Widened IVD L5-L6	Narrowed IVD L6-S1 Severe periarticular bone proliferation BL SI joint
3	Moderate periarticular bone proliferation AP C2-C4	Moderate SP impingement T16-T17 Moderate periarticular bone proliferation AP T16-T17 Moderate SP impingement L5-L6	Moderate periarticular bone proliferation L6-S1
4	Mild IVDD C3-C5 Moderate IVDD C6-C7 Severe IVDD C7-T1 Hemorrhage C7-T1	NE	NE
5	Moderate periarticular bone proliferation AP C6-T1 Thickened joint capsule AP C3-T1 Severe IVDD C7-T1	Moderate IVDD T3-T4	Severe periarticular bone proliferation BL SI joint
6	Moderate periarticular bone proliferation AP C2-T5 Severe periarticular bone proliferation AP C7-T1	Impinged SP T11-T17	Osteochondroma right ilium
7	Severe IVDD C6-T1 Thickened joint capsule right AP C6-C7 Moderate IVDD C5-C6 Moderate periarticular bone proliferation AP C6-T1	Dural hemorrhage T1-T2 Moderate IVDD T1-T2 Moderate periarticular bone proliferation IT joint L4-L5	Severe periarticular bone proliferation BL SI joint Hemorrhage LS

		Severe periarticular bone proliferation IT joint and IVF occlusion L5-L6 Ankylosis L5-L6	
8	Moderate periarticular bone proliferation AP C3-C5, C6-T1 Thickened joint capsule AP C3-C5, C6-T1 Malformation C3-C4 (pseudoarthrosis) Hemorrhage C2-C3	NE	NE
9	Moderate IVDD C2-C4 Severe IVDD C7-T1 Moderate periarticular bone proliferation AP C3-C6	Moderate IVDD T3-T4 Ankylosis IT joint L5-L6	Moderate periarticular bone proliferation and IVF occlusion L6-S1 Moderate periarticular bone proliferation right SI joint
10	NSF	Dural hemorrhage T4-T9 Spondylosis L3-L4 Narrowed IVD L5-L6 Ankylosis L5-L6	Moderate periarticular bone proliferation and IVF occlusion L6-S1 Stress fracture left L6 Severe periarticular bone proliferation left SI joint Dural hemorrhage L6-S1 and cauda equina
11	Hemorrhage right poll Dural hemorrhage C1-C4 Moderate periarticular bone proliferation AP C2-C4 Severe IVDD C4-T1 Thickened joint capsule AP C6-T1	Moderate periarticular bone proliferation right IT joint L5-L6 Severe IVDD L5-L6 Severe protrusion IVD L5-L6	Sacralization of L6 Severe periarticular bone proliferation BL SI joints Dural hemorrhage L6-S1 and cauda equina
12	Moderate periarticular bone proliferation left AP C7-T1	Moderate periarticular bone proliferation Right AP L5-L6 Epidural hemorrhage T1-T3	Moderate periarticular bone proliferation BL SI joints Sacralization of L6
13	Moderate IVDD C3-C6 Moderate periarticular bone proliferation BL AP C2-C7 Severe periarticular bone proliferation right AP C5-C6	Dural hemorrhage L4-S1	NSF
14	Moderate periarticular bone proliferation AP C6-C7	Mild periarticular bone proliferation IT joint L4-L5 Moderate periarticular bone proliferation IT joint L5-L6	Sacralization of L6

Table 5. Histopathologic findings across spinal regions. NE = Not examined, C = Cervical vertebrae, T = Thoracic vertebrae, LS = Lumbosacral joint (L6-S1), S = Sacral vertebrae.

Horse	Cervical	Thoracolumbar	Lumbosacral and Sacropelvic
1	Moderate ganglionitis C4-C6	Severe ganglionitis T9-T18	Moderate ganglionitis LS Epidural hemorrhage LS
2	Moderate ganglionitis C4-T1 Epidural hemorrhage C7-T1	Epidural hemorrhage T1-T2	NE
3	Severe ganglionitis C2-T1	Moderate ganglionitis T1-T4, T17-T18 Severe ganglionitis T11-12	NE
4	Moderate ganglionitis C1-C6 Severe ganglionitis C6-C7 Epidural hemorrhage C7-T1	Severe ganglionitis T1-T2	NE
5	Severe ganglionitis C6-T1 Left brachial plexitis	Severe ganglionitis T2-T3	NE
6	Severe ganglionitis C1-C2, C7-T1	NE	NE
7	Moderate ganglionitis C3-C6 Severe ganglionitis C7-T1 Left brachial plexitis	Severe ganglionitis T1-T3 Epidural hemorrhage T5	Severe ganglionitis LS Perineural hemorrhage LS
8	Moderate ganglionitis C1- T1	Severe ganglionitis T2-T3 Subdural hemorrhage T2-T3	NE
9	Moderate ganglionitis C3-C5, C6-T1 Epidural hematoma C7-T1	Moderate ganglionitis T1-T2	NE
10	Moderate ganglionitis C3-C5, Severe ganglionitis C7-T1	Severe ganglionitis Left T1-T5 Epidural hemorrhage T3-T5	Subdural hemorrhage LS
11	Moderate ganglionitis C1-C3, C5-C7 Severe ganglionitis C7-T1 Epidural hematoma C1-C4	NE	Moderate ganglionitis LS Hemorrhage sacral nerve roots
12	Severe ganglionitis C4-T1	Severe ganglionitis T1-T5 Moderate ganglionitis Right L4-L5	NE
13	Moderate ganglionitis C4-C5, C6-C7 Bilateral brachial plexitis	NE	NE
14	Severe ganglionitis C6-C7 Moderate ganglionitis C3-C4, C7-T1 Right brachial plexitis	Severe ganglionitis T1-T2	Severe ganglionitis S3 Epidural hemorrhage S2-S3

Table 6. Compiled clinical case summaries. The most severe findings localized to an affected spinal region are listed for the spinal examination, diagnostic imaging and pathology examinations with a final aggregate assessment across columns of the primary spinal region judged to be causing the dangerous pain behavior. IVD = Intervertebral disc, IVDD = Intervertebral disc degeneration, NE = Not examined, NSF = No significant findings, BL = Bilateral, SI = Sacroiliac.

Horse	Spinal Exam	Imaging	Gross Pathology	Histopathology	Aggregate
1	Thoracolumbar Lumbosacral	Cervical Cervicothoracic Thoracolumbar Lumbosacral Sacroiliac joint	Lumbosacral	Thoracic Lumbosacral	**Lumbosacral:** Narrowed vertebral canal and IVD protrusion L6-S1, dural and epidural hemorrhage L6-S1
2	Cervicothoracic	Cervical Cervicothoracic	Cervical Cervicothoracic Thoracolumbar Lumbosacral Sacroiliac joint	Cervicothoracic	**Cervicothoracic:** Severe IVDD C4-T1 with dorsal protrusion C6-T1, dural and epidural hemorrhage C7-T1
3	Cervical Lumbosacral	Cervical	Cervical Thoracolumbar Lumbosacral	Cervical Cervicothoracic Thoracic	**Lumbosacral:** Moderate periarticular bone proliferation L6-S1
4	Cervical	Cervical	Cervicothoracic	Cervical Cervicothoracic	**Cervicothoracic:** Severe IVDD C7-T1, severe ganglionitis C6-C7, epidural hemorrhage C7-T1
5	Cervicothoracic Thoracolumbar	Lumbar	Cervicothoracic Sacroiliac joint	Cervical Cervicothoracic Brachial plexus	**Cervicothoracic:** Severe IVDD C7-T1, severe ganglionitis C6-T3, Brachial plexitis
6	NE	NSF	Cervical Cervicothoracic Thoracolumbar	Cervical Cervicothoracic	NE
7	Cervicothoracic Sacropelvic	Cervical Sacroiliac joint	Cervicothoracic Lumbar Lumbosacral Sacroiliac joint	Cervicothoracic Brachial plexus Thoracic Lumbosacral	**Cervicothoracic**: Severe IVDD C6-T1, severe ganglionitis C7-T1, dural hemorrhage T1-T2, brachial plexitis
8	Cervical Cervicothoracic	Cervical	Cervical	Cervicothoracic	**Cervicothoracic:**

					Severe ganglionitis T2-T3, subdural hemorrhage T2-T3
9	Cervical Thoracolumbar Sacropelvic	Cervical Lumbosacral Sacroiliac joint	Cervical Cervicothoracic Lumbar Lumbosacral Sacroiliac joint	Cervical Cervicothoracic	**Cervicothoracic:** Severe IVDD C7-T1, epidural hematoma C7-T1
10	 Cervicothoracic Thoracolumbar Sacropelvic	Cervical Cervicothoracic	 Thoracic Lumbar Lumbosacral Sacroiliac joint	 Cervicothoracic Thoracic Lumbosacral	**Cervicothoracic:** Severe ganglionitis C7-T5, epidural hemorrhage T3-T5
11	Cervical Lumbosacral	Cervical Lumbosacral	Cervical Cervicothoracic Lumbar Lumbosacral Sacroiliac joint	Cervical Cervicothoracic Sacral	**Lumbosacral:** Sacralization of L6, severe periarticular bone proliferation BL SI joint, dural hemorrhage L6-S1, hemorrhage sacral nerve roots
12	 Cervicothoracic Thoracolumbar Lumbosacral	 Cervicothoracic Lumbosacral	 Cervicothoracic Lumbar Lumbosacral Sacroiliac joint	Cervical Cervicothoracic	**Lumbosacral**: Sacralization of L6
13	Cervical	Cervical	Cervical Lumbosacral	Cervical Brachial plexus	**Cervical:** Severe periarticular bone proliferation C5-C6
14	 Lumbosacral	Cervical Lumbosacral	Cervical Lumbar Lumbosacral	Cervical Cervicothoracic Brachial plexus Sacral	**Lumbosacral:** Sacralization of L6, severe ganglionitis S3, epidural hemorrhage S2-S3
Totals	Cervical = 6 Cervicothoracic = 6 Thoracolumbar = 5 Lumbosacral = 5 Sacropelvic = 2	Cervical = 11 Cervicothoracic = 4 Thoracolumbar = 1 Lumbosacral = 5 Sacropelvic = 3	Cervical = 8 Cervicothoracic = 8 Thoracolumbar = 3 Lumbar = 6 Lumbosacral = 10 Sacropelvic = 7	Cervical = 9 Cervicothoracic = 12 Thoracic= 4 Lumbosacral = 3 Sacropelvic = 2	Cervical = 1 Cervicothoracic = 7 Lumbosacral = 5

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CHAPTER 3:
USE OF INTERLEUKIN-1 BETA AS AN INTRAARTICULAR MODEL OF ACUTE NECK PAIN IN HORSES

Introduction

Reduced performance secondary to neck pain and dysfunction is a complex topic that has gained much attention recently (1-3). Osteoarthritis (OA) of the cervical APJ has been reported to cause signs of neck pain and stiffness (4), forelimb lameness (5), and is considered a potential contributing factor to unwanted or dangerous behavior (6). However, enduring controversies exist regarding the clinical significance of osseous changes localized to the cervical APJs identified on radiographic imaging as there is no clear association with the presence and severity of clinical signs (2, 7, 8). Unfortunately, clinical significance is often attributed only to the presence of radiographic changes without clear localization based on detailed spinal evaluation or diagnostic analgesia. As the clinical signs attributed to neck pain and stiffness vary widely (4), it is often difficult to establish a definitive diagnosis. As an adjunctive diagnostic imaging modality, ultrasonography of the cervical region is commonly used to identify soft tissue changes (e.g., joint effusion, joint capsule thickening) and osseous proliferation of the APJ margins (9-11). However, there is also a persistent knowledge gap regarding the clinical significance of abnormal ultrasonographic findings within the cervical region of horses. Within the distal limbs, diagnostic local anesthesia is used to elucidate the clinical relevance of observed clinical signs and abnormal diagnostic imaging findings. Within the cervical region there are a few reports of using diagnostic local anesthesia to localize sites of pain or upper forelimb lameness (12, 13); however, many practitioners are not comfortable with injecting local anesthetics into the cervical

APJ due to perceived risks of affecting neurovascular tissues (e.g., brachial plexus) or the spinal cord.

Localized pain, soft tissue swelling, and joint effusion are typically associated with synovitis in the appendicular skeleton (14). Within the cervical region, the APJs are often difficult to visualize or palpate due to their deep location and overlying musculature, which contributes to a relative lack of knowledge of the clinical signs associated with cervical OA or synovitis. Detailed palpation of the cervical region to identify sites and the severity of pain, stiffness, and muscle hypertonicity has provided valuable clinical information in affected horses; however, not all practitioners are trained or highly-skilled in using these techniques or interpreting the findings (6). Given the large between-horse variability in clinical signs (4) and differences in perceived clinical relevance of cervical pain and dysfunction, there is a critical need to develop uniform, spinal examination procedures.

The use of inertial sensors and biomechanical approaches to evaluate spinal kinematics have been used in horses (15, 16). The use of induced back-pain or limb-lameness models have helped to better understand appendicular-axial skeleton interactions (17). The pathogenesis of neck pain and dysfunction, and its effect on local and compensatory gait mechanisms is poorly understood. The development of an acute, temporary neck pain model that could be used to monitor changes in nociception, stiffness, locomotion, and diagnostic imaging would provide a significant advancement in assessing the presence and clinical significance of neck-related issues in horses.

Recombinant equine interleukin-1β (reIL-1β) has been used as a model of acute synovitis induction within the appendicular skeleton to explore the pathogenesis, clinical signs, and treatment of early joint disease development in horses (18-21). Applying this acute synovitis model to the cervical APJs was expected to provide novel clinical and diagnostic information related to the development of neck pain in horses. The objective of this study was to develop an acute neck pain model using reIL-1β to induce transient synovitis within the APJs of horses. We hypothesized that reIL-1β injected into a unilateral C5-C6 APJ will produce acute synovitis and clinical signs of neck pain that will be identified on clinical, biomechanical, and ultrasonographic examinations.

Materials and Methods

Horses

Twelve clinically-normal adult horses were evaluated for evidence of neck pain for consideration of inclusion into the study. Cervical radiographs were acquired to rule out the presence of APJ osseous changes that would be indicative of OA. Horses were excluded if clinically significant abnormalities were noted on cervical, lameness, or radiographic examinations. The study was approved by the Institutional Animal Care and Use Committee (IACUC) at Colorado State University (#16-6681A).

Induction of Synovitis

Within horse, reIL-1β (R&D Systems-Cat#3340-EL) injections were randomized to either the left or right C5-C6 APJs while the contralateral articulation was injected with 1 ml of sterile phosphate buffered saline. The dosage of reIL-1β was extrapolated from prior studies evaluating

induced synovitis within appendicular articulations (19, 21). In the first six horses, 50 ng of reIL-1β was used; however, there was inconsistent evidence of induced APJ effusion noted on ultrasonography. Therefore, 100 ng of reIL-1β was administered in the remaining six horses.

Clinical Examination

Based on time points reported in reIL-1β synovitis models in appendicular joints (19, 21, 22), horses were evaluated for all outcome parameters at 0, 4, 8, 24, 48 hours and 7- and 14-days post injection. Digital palpation of the superficial soft tissues and muscles of the cervicothoracic region (C1-T8) was performed to assess myofascial tone and the response to applied pressure. The cervical region was also assessed for the quality of induced passive joint motion in lateral bending (23). Responses to the myofascial examination and joint mobilization were graded as normal (0, no response), mild (1), moderate (2), and severe (3) avoidance reactions or stiffness. The clinical examination score was calculated as the cumulative unilateral score of neck pain and stiffness.

Ultrasonographic Examination

Ultrasound examination (GE Healthcare, Logiq 90, 12 MHz linear probe, or the Toshiba Aplio i700, 10MHz linear probe, based on availability of ultrasound machine) of the bilateral cervical C5-C6 APJs was performed by a board-certified radiologist (MB). Stored ultrasonographic images were evaluated and graded by a blinded, board-certified equine surgeon and sports medicine specialist (MS). APJ effusion was scored as none (0), mild (1), moderate (2), moderate-to-severe (3), and a severe (4) increase in the observed volume of joint fluid and capsular distension. Horses that had moderate to severe joint effusion (grade 2, 3, or 4) at any

time point were classified as “responders”. Horses that had only grade 0 or 1 joint effusion were considered non-responders.

Gait Evaluation

Gait evaluation included lameness and neurologic evaluations. The neurologic examination included walking in a straight line with the head in a neutral and elevated position, walking in small circles to the left and right to evaluate forelimb and hindlimb placement, backing the horse up for several strides, and evaluating the response to lateral tail traction at a stance and during walking to assess signs of paresis. Ataxia was graded 0-5 based on the modified Mayhew scale (24). For objective lameness evaluation, each horse was instrumented with a wireless, inertial sensor-based motion analysis system (Equinosis Q, Lameness Locator). The inertial sensor system consisted of two uniaxial accelerometers attached to the dorsal midline of the poll and pelvis and a uniaxial gyroscope attached to the right front pastern according to manufacturer’s recommendations. The inertial sensor data was collected at 200 Hz and analyzed using proprietary software. Horses were evaluated at the trot in a straight line for 5 trials that had at least 25 strides per trial. Data from two consecutive, stable trials were averaged at each time point. Variables examined included the maximum and minimum difference in head position (HDMax and HDMin, respectively). The vector sum for the forelimbs was calculated using HDMax and HDMin to provide an overall measurement of forelimb lameness (25).

Kinetic Analysis

Ground reaction forces (GRF) for all four limbs were recorded using two strain-gauge based force platforms (60 x 90-cm) mounted serially in an isolated concrete base in the center of a 25-

m runway. Each horse was led by an experienced handler in a straight line over the force platforms at a consistent trotting velocity (2.8-3.3 m/s). A trial was considered successful when the ipsilateral thoracic and pelvic limb pairs contacted the center of a single force platform. Orthogonal GRF data was sampled at 2000 Hz and the vertical and craniocaudal GRFs of the forelimbs were analyzed for stance duration, peak vertical, braking, and propulsive forces and impulses. Kinetic variables were averaged across the five trials for each time point and normalized to subject body mass and reported as N/kg or Ns/kg.

Kinematic Analysis

Optical data was collected at 250 Hz using a motion analysis system with twelve high-speed infrared cameras (Qualysis) distributed equally around the periphery of the force platforms runway. The capture volume over the force platforms was calibrated using a customized calibration frame and wand with an accuracy of 0.7 mm. The hair was clipped to ensure consistent marker placement throughout the study. Cyanoacrylate glue was used to adhere 2.5-cm spherical retro-reflective markers to the skin overlying anatomical landmarks of the head, cervical spine, and thoracic limbs. Markers were placed over the bridge of the nose, the left and right transverse processes of the cervical vertebrae (C1-C6), and the dorsal midline on the T5 spinous process (Figure 2). Intersegmental angles between adjacent cervical segments were measured based on flexion-extension planar angles. The range of motion of the C5-C6 intervertebral joint was calculated using the C1-C6-T5 marker set. The vertical displacement of the head was calculated by the distances from the left and right C1 marker, relative to the T5 marker. Raw coordinate data was filtered with a low-pass fourth-order recursive Butterworth filter at 12 Hz. Kinematic variables calculated for each trial included maximum, minimum, and range of motion (max-min) for the joint angles and vertical displacements during the stance and swing phases of each stride.

Data was averaged over 5 trials. The joint angles were reported as angular changes from those measured during the square stance position and defined as positive values (flexion) or negative (extension) values. Joint angle tracings were normalized to the duration of the entire stride and divided into stance to swing phases.

Statistical Analysis

Sample size estimates and the concentration of reIL-1β were based on reported effect sizes and variances produced using this model within appendicular articulations (19, 20). JMP (JMP Pro 15) was used for all statistical testing with $p < 0.05$ considered significant. A mixed model was fit separately for each response variable. Model assumptions were assessed by visual inspection of diagnostic plots. Fixed effects included treatment (saline, reIL-1β 50 ng, and reIL-1β 100 ng), time (0, 4, 8, 24, 48 hours, 7 and 14 days) and treatment*time interactions. Horse was included as a random effect to account for repeated measures within horses. Analysis of Variance (ANOVA) F-tests were evaluated for main effects and interactions. Contrast style F-tests were used to compare treatments at each time point, and to compare time points for each treatment using JMP Test Slices option (equivalent to SAS slice command). Individual comparisons were made using contrasts and pairwise-comparisons when indicated.

Results

Horses

Twelve horses were enrolled in the study that consisted of eight mares and four geldings, with an average age of 5.3 ± 2.5 years, and body weight of 393 ± 52 kilograms. No adverse events occurred during or after the saline or reIL-1β injections.

Clinical Examination

There was no significant difference in the clinical examination scores (i.e., myofascial palpation and cervical mobilization) at any time point (Figure 3). There was a main effect of time ($p < 0.0001$), which peaked at 24 hours within the saline and reIL-1β 100 ng groups, and peaked at 48 hours with the reIL-1β 50 ng group.

Ultrasonographic Examination

There was a significant increase in APJ effusion noted on ultrasonography at 8, 24, and 48 hours regardless of the dose of reIL-1β administered (Figures 4 and 5). Six horses treated with reIL-1β (two with the 50 ng dose, four with the 100 ng dose) continued to have mild joint effusion at 14 days. Seven horses developed moderate to severe joint effusion (i.e., responders) at one or more time points (Figure 6). Of these, four horses received 50 ng reIL-1β and three horses had received 100 ng reIL-1β.

Gait Evaluation

No horses developed any signs of neurologic deficits at any time point in the study. An increase in lameness was noted using the inertial sensor system at 8 hours in the horses receiving 100 ng reIL-1β, compared to saline or 50 ng reIL-1β (Figure 7). At 48 hours, there was a significant decrease in lameness in those horses that received 50 ng reIL-1β, compared to saline.

Kinetic Analysis

There were no significant differences found in peak vertical forces between groups at any time point or within a group over time. There was a significant difference in the peak breaking force at 4 hours ($p = 0.004$) and 7 days ($p = 0.016$) in the horses that received 100 ng reIL-1β. However, on further evaluation, these results are due to positive braking forces within a single horse at both time points.

Kinematic Analysis

There was a main effect of time ($p = 0.003$) on the angle measured from C1-C6-T5 during the stance phase, but not the swing phase (Table 7). The was no difference in vertical displacement of the head during the stance or swing phases of stride.

Discussion

We were able to induce synovitis within the cervical APJ, as evidenced by the presence of joint effusion, and signs of acute neck pain using both 50 ng and 100 ng of reIL-1β. Ultrasonographic examination provided a sensitive tool for grading of the presence and severity of joint effusion, which peaked at 24 hours and persisted for 14 days. The intraarticular injection of reIL-1β into a known location within the cervical region provided the opportunity to evaluate the onset and development of clinical signs and associated biomechanical outcome parameters. The caudal cervical region is frequently implicated as a common site of OA and pain; therefore, we chose the C5-C6 vertebral level as a clinically relevant location to simulate the clinical condition. Chronic OA of the cervical APJs has been implicated as a cause of forelimb lameness (4). In the current study, increased ipsilateral forelimb lameness was noted at 8 hours post-injection in

horses that received 100 ng of reIL-1β. This suggests that horses may alter their cervicothoracic biomechanics in an effort to guard the local site of neck pain and synovitis (i.e., chemically-mediated), which can affect measures of forelimb lameness. This contrasts with the general belief that in horses with poorly localized forelimb lameness and concurrent neck pain that the primary etiology is severe APJ OA or cervical radiculopathy due to mechanical compression or occlusion of the adjacent intervertebral foramen (5, 13).

Kinematic studies of the cervical spine have been reported in cadaveric specimens (26) and normal adult horses (27). To the authors knowledge, there are no studies that have evaluated the kinematic effects of induced cervical pain; as has been reported within the thoracolumbar region (17). In our study, decreased cervical mobility occurred at 4 hours as measured by the C1-C6-T5 angle. While there was no significance found between groups, there was a main effect of time, similar to the cervical examination scores. This again suggests that there was not an effect only on the reIL-1β side of the neck, but a regional effort to protect or restrict motion within the entire neck. This is similar to the clinical presentation of neck pain, where unilateral localization may be difficult. While the kinematic and kinetic measures of neck and forelimb biomechanics did not provide the expected results, it is likely that the incorporation of an arena-based kinematic assessment of horses walking, trotting, and cantering on a circle or while turning would provide much more useful information related to the biomechanical assessment of induced neck pain and associated forelimb lameness.

The prescribed dose, selected joint, inter-subject variability, and the onset and duration of joint effusion are all factors that need to be closely considered in studies using reIL-1β as an acute

synovitis model in horses. Within the middle carpal joint 100 ng of reIL-1β is reported to be an effective dose for producing transient synovitis (18, 19, 28). However, other studies have used variable dosages of reIL-1β within other appendicular articulations due to noted differences in the onset and duration of synovial effusion and degree of induced limb lameness (20, 22, 29). In the current study, APJ effusion was the most readily identified clinical sign observed. There also appears to be a large between-subject variability in the response to reIL-1β injections. We found that 42% (5 of 12) of horses were considered 'nonresponders', while 58% (7 of 12) developed a moderate to severe increase in APJ effusion, regardless of the dose of reIL-1β administered. The authors acknowledge that pilot work is needed to select an appropriate reIL-1β dose to use in an untested articulation. Based on our results, we recommend a single dose of 100 ng of reIL-1β to be used within the cervical APJ.

We did not evaluate synovial fluid cell counts, protein concentration, or biomarkers (e.g., Prostaglandin E_2 (PGE2), Matrix Metalloproteinase (MMP) activity) (19, 22) as our focus was on global measures of neck pain and the associated effects on clinical, biomechanical, and ultrasonographic examinations. The lack of a true control horse population is a limitation of this study. While this study did have a treated and a control side, and paired articulations within both fore and hind limbs have been used as controls previously (21), the use of intraarticular reIL-1β within the cervical region may necessitate the use of a separate control group or a cross-over designed study to compare outcomes in horses with and without induced neck pain. Horses had bilateral reactivity to palpation and mobilization that could not be isolated to the specific saline and reIL-1β injections sites. Given that the intervertebral segments are linked biomechanically (i.e., a three-joint complex), and independent motion of the left versus right sides of the C5-C6

APJs is not possible, incorporating a “whole horse” control may be a consideration in future studies.

Conclusion

Given the limitations of this study, we were able to gain useful information about the utility of reIL-1β as a model of acute neck pain and synovitis in horses. Ultrasonographic evidence of the presence and severity of APJ effusion could be readily identified and tracked over time. Acute synovitis of the APJ induced clinical signs of myofascial pain and neck stiffness with variable degrees of forelimb lameness.

Contributions

All authors contributed to the research project’s design and conception; data analysis and interpretation; and in the writing, revising and final approval of the manuscript.

Role of the funding source

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Conflict of interest

None of the authors have conflicts of interest related to the manuscript.

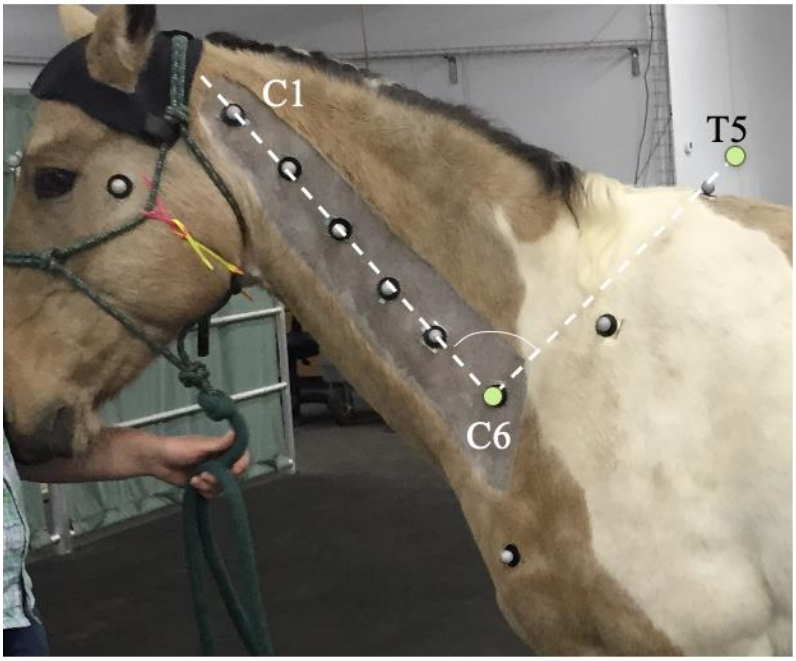

Figure 2. Photograph of the marker set used in the study to measure head and neck mobility during kinematic analysis. Note the dorsal angle at the C6 marker formed by the C1-C6-T5 markers.

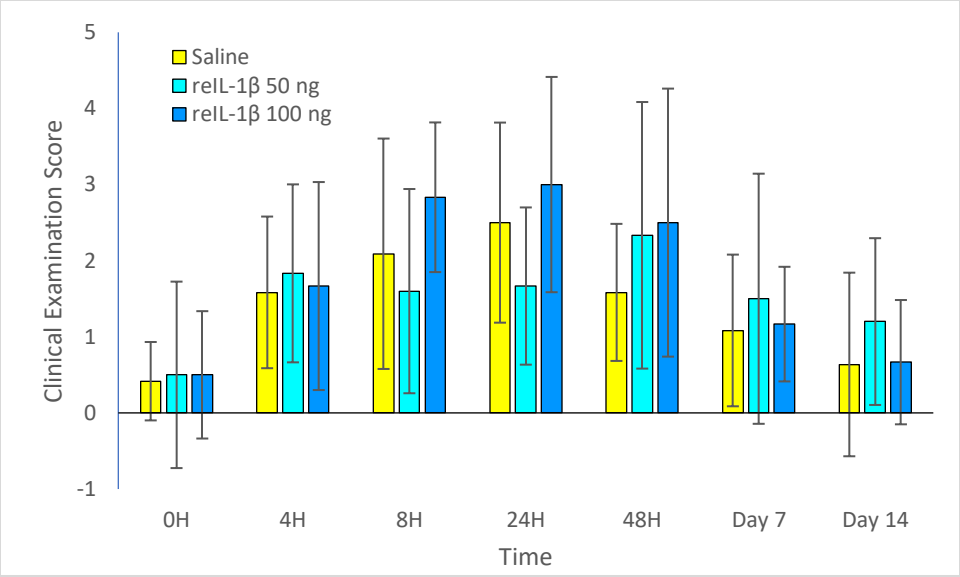

Figure 3. There was a main effect of time for the clinical examination scores ($p < 0.0001$) across the cervicothoracic region (C1-T8) on the side of the saline and reIL-1β injections at the C5-C6 APJ. The score peaked at 24 hours for the saline and reIL-1β 100 ng injections, and peaked at 48 hours for the reIL-1β 50 ng injections. Grades used: normal (0, no response), mild (1), moderate (2), and severe (3) avoidance reaction.

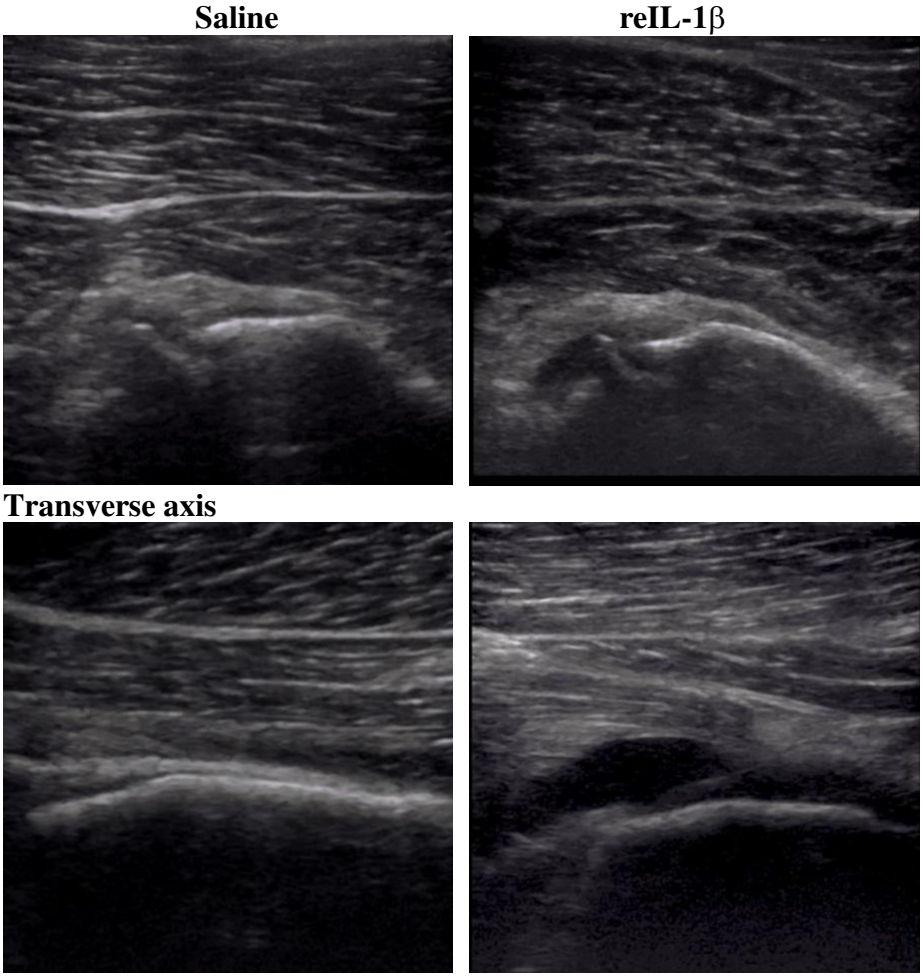

Figure 4. Transverse (top panels) and longitudinal (bottom panels) ultrasonographic images of the C5-C6 APJ 24 hours post injection with saline (left panels) and reIl-1β (right panels). Note that there is minimal joint effusion at the saline site; whereas, there is severe (grade 4) joint effusion present at the reIL-1β site.

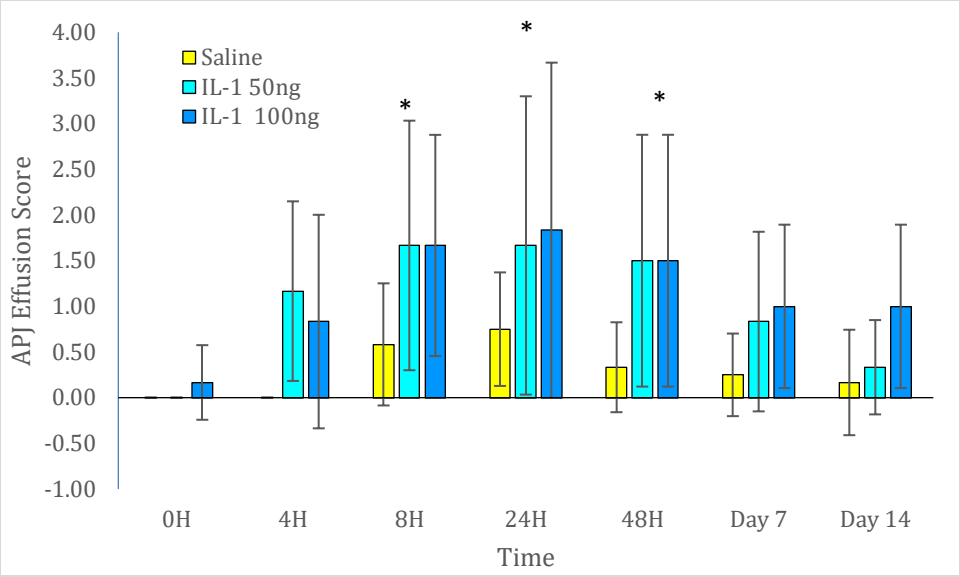

Figure 5. APJ effusion scores at C5-C6 as diagnosed on ultrasonography. * Indicates significant differences (all $p < 0.014$) between saline versus reIL-1β 50 ng or reIL-1β 100 ng. There was no significant difference between reIL-1β 50 ng and reIL-1β 100 ng at any timepoint. Grades used: none (0), mild (1), moderate (2), moderate-to-severe (3) and severe (4) joint effusion.

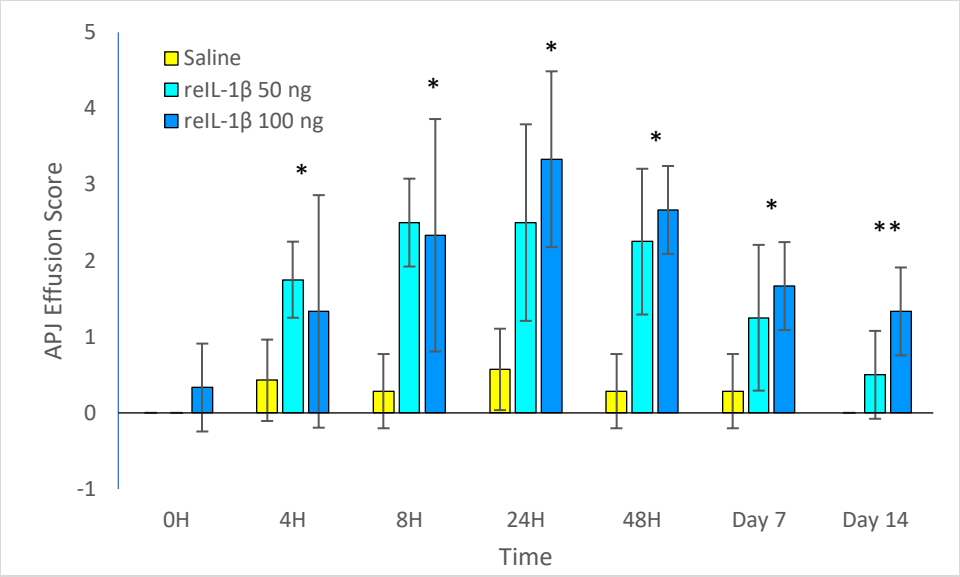

Figure 6. Ultrasound effusion scores of the articular process joints at C5-C6 in the horses classified as responders to the reIL-1β regardless of the dosage. * Indicates significant differences (all $p < 0.04$) between saline and reIL-1β 50 ng and 100 ng. ** Indicates significant difference ($p = 0.002$) between saline and reIL-1β 100 ng.

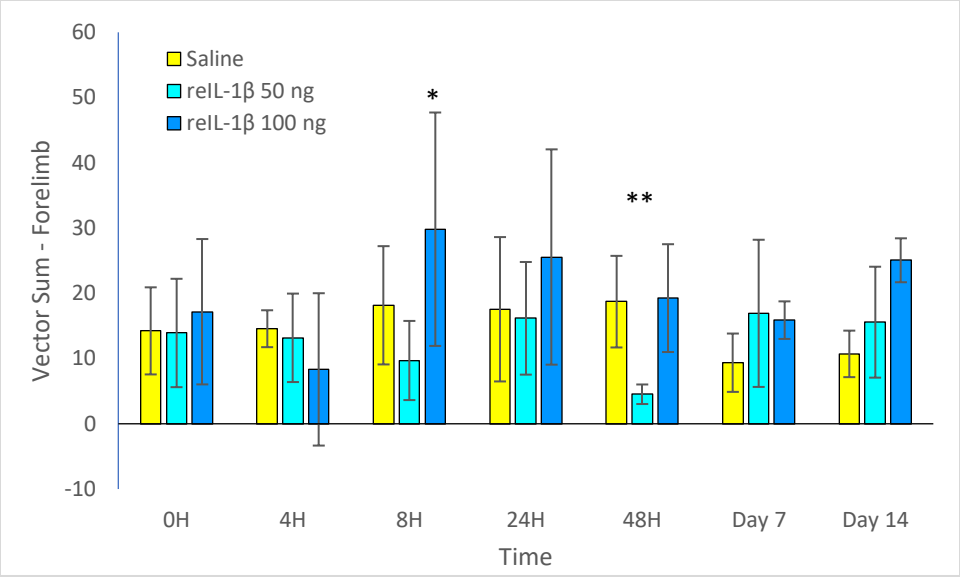

Figure 7. Vector sum values used as a measure of forelimb lameness. * Indicates significant increase in lameness between reIL-1β 100 ng and saline ($p = 0.013$) or reIL-1β 50 ng ($p = 0.001$). ** Significant decrease in lameness in reIL-1β 50 ng ($p = 0.004$) group.

Table 7. Cervical angle measured at C1-C6-T5 during the stance phase (in degrees). There was a main effect of time (p = 0.003) in the cervical angle at C6, stance phase. Regardless of treatment; there was a smaller, though not significant, angle at C6 at 4 hours (highlighted) post injection.

Group	**0 H**	**4 H**	**8 H**	**24 H**	**48 H**	**Day 7**	**Day 14**	*p-values*
Saline	7.3 ± 1.2	6.5 ± 1.8	7.5 ± 2.1	7.0 ± 1.9	6.7 ± 1.7	6.7 ± 1.4	6.6 ± 1.7	0.203
IL1-β 50	7.7 ± 2.6	6.0 ± 1.6	7.5 ± 2.1	7.0 ± 1.7	6.3 ± 1.6	6.7 ± 2.1	6.1 ± 1.4	**0.049**
IL1-β 100	7.8 ± 1.2	6.7 ± 1.4	7.0 ± 1.6	7.2 ± 1.6	6.8 ± 1.2	7.0 ± 0.9	7.0± 0.9	0.646
p-values	0.591	0.820	0.417	0.963	0.918	0.930	0.687	

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SUMMARY AND FUTURE DIRECTIONS

This body of work highlights the clinical importance of equine neck pain and dysfunction. The collective material also opens the door for a more in-depth understanding of pain behaviors and the impact of neuropathic pain in horses. Moving forward, there are opportunities to provide a deeper understanding of the clinical presentation of these painful horses in order to recognize prodromal signs earlier in the disease process. In addition, expanding our knowledge of the cellular and molecular mechanisms that contribute to neuropathic pain will offer new treatment approaches to provide affected horses with a comfortable and productive life.

To provide a foundational basis of the current understanding of neck pain and dysfunction, a review of the current literature addressed the clinical presentation, pathophysiology, diagnostic capabilities, and treatment options in horses. There is growing interest and awareness of axial pain syndromes that are driving an expanded knowledge base. Therefore, this review serves as a starting point, as there is a consistent flow of available new information.

A clinical case series of horses that presented with dangerous behavior attributed to neck pain emphasizes the need for improved recognition of painful behaviors and their clinical presentation. This work included detailed spinal and neurologic examinations, extensive diagnostic imaging and gross and histopathologic evaluations. While all horses presented for suspected neck pain, further evaluation uncovered a systemic pain syndrome with cellular infiltration of the dorsal root ganglia. Ganglioneuritis has been associated with neuropathic pain syndromes and was recognized in all horses included in this case series.

Identification of the exact source of neck pain is challenging at best. Therefore, there is a critical need to develop an acute neck pain model to elucidate the clinical signs associated with APJ pain, which is a commonly reported source of cervical pathology in horses. This work incorporated reIL-1ß as an acute synovitis model within a single cervical APJ that was readily identified on ultrasonographic examinations. Study limitations provided valuable insights into necessary changes in the pain model application and needed outcome measures to more fully understand how horses respond and adapt to a known source of cervical pain.

Looking ahead, many unanswered questions require focused avenues of investigation. Based on novel discoveries identified in this body of work, it has been challenging to find the expertise to help uncover the underlying cellular and molecular mechanisms thought to be responsible for the histopathologic findings noted. Fortunately, there is now a group of collaborators in place, with exciting work planned.

From a clinical perspective, the reIL-1ß model will allow further studies to investigate the early pain pathways and mechanisms that lead to the development of dorsal root ganglionitis. The continued collection of horses with clinical signs of neck pain and dysfunction provides important case material for immunohistochemistry of ganglionitis and the newly discovered increase in sympathetic terminals surrounding medium and large diameter neuronal cell bodies. These sympathetic nerve terminals form perineuronal rings, or baskets, and have been reported in several chronic pain models. In neuropathic pain models, these baskets surrounding medium and large diameter neurons show spontaneous activity and may contribute to hypersensitivity or

spontaneous pain. It is suspected that mechanical afferent signals are modified and perceived as painful stimuli due to sprouting that preferentially forms around mechanoreceptors. Calcitonin-gene-related peptide (CGRP), which has been linked to chronic neuropathic pain, shows an apparent increase in the affected horses. There is also a distinct distribution of macrophage and satellite glia in affected horses' dorsal root ganglia (DRG).

While we have multiple markers indicating neuropathic pain in the horses in our study, there are still many unanswered questions. Utilizing technology that allows for high-throughput analysis of gene expression will allow us to screen for hundreds of genes to search for yet unknown critical factors sending these animals down the neuropathic pain route. We are in a unique position to benefit the horse and potentially answer questions of neuropathic pain mechanisms for many species. We can study this disease in a naturally occurring clinical setting. Not only do we have the potential to define the pathways and progression, we then have greater potential to develop novel treatments to allow for a pain-free life. This work sets the stage not only to benefit horses, but also for powerful translational impact, not only within veterinary medicine, but human medicine as well.