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Review Article

State-of-the-Art Diagnostic Methods to Diagnose Equine Spinal Disorders, With Special Reference to Transcranial Magnetic Stimulation and Transcranial Electrical Stimulation

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ABSTRACT

Spinal cord disorders are a common problem in equine medicine. However, finding the site of the lesion is challenging for veterinarians because of a lack of sensitive diagnostic methods that can assess neuronal functional integrity in horses. Although medical imaging is frequently applied to help diagnose corticospinal disorders, this approach does not reveal functional information. For the latter, transcranial magnetic stimulation (TMS) and more recently transcranial electrical stimulation (TES) can be useful. These are brain stimulation techniques that create either magnetic or electrical fields passing through the motor cortex, inducing muscular responses, which can be recorded either intramuscularly or extramuscularly by needle or surface electrodes. This permits the evaluation of the functional integrity of the spinal motor tracts and the nerve conduction pathways. The interest in TES in human medicine emerged these last years because unlike TMS, TES tends to bypass the motor cortex of the brain and predominantly relies on direct activation of corticospinal and extrapyramidal axons. Results from human medicine have indicated that TMS and TES recordings are mildly if not at all affected by sedation. Therefore, this technique can be reliably used in human patients under either sedation or full anesthesia to assess functional integrity of the corticospinal and adjunct motor tracts. This opens important new avenues in equine medicine.

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1. Introduction

Spinal cord pathologies, for example, compression due to an external cause or infection, are frequently encountered in horses [1–3], but their diagnosis remains challenging because of a lack of sensitive diagnostic methods that can assess neuronal functional integrity in horses. The common diagnostic tools are a thorough clinical and neurological examination combined with radiography, ultrasound, myelography, scintigraphy, computed tomography, and/or magnetic resonance imaging. Apart from the clinical examination and medical imaging, cytological, biochemical, and immunological analysis of cerebrospinal fluid may further assist in diagnosis.

Recently, it has been reported how the anatomical structures of the cervical and lumbosacral vertebral canal can be visualized in horses by means of epiduroscopy and myeloscopy. This approach allows us to precisely determine the exact location of spinal cord compression [4–6]. However, important risks are associated with these techniques, such as retinal hemorrhage [7], encephalopathies, and rhabdomyolysis [8].

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In the last two decades, two promising "functional" neurological diagnostic techniques have been developed: transcranial magnetic stimulation (TMS) and, more recently, transcranial electrical stimulation (TES). Both techniques are used to evaluate the functional integrity of the descending spinal motor tracts. Both TES and TMS can even have therapeutic potential because they are selective and noninvasive methods of neurostimulation, which can induce long-term cortical changes if the stimulation lasts sufficiently long [9]. While TMS was broadly used in human medicine for diagnostic purposes, TES was cast aside for many years because it was considered to be a painful and discomforting technique. However, TES appears to be more reliable, accurate, and less sensitive to sedative effects than TMS to monitor spinal cord functions [10,11]. The interest in TES revived and the number of studies and new applications of this technique seem to expand steadily [10,12–14]. In the recent history of motor monitoring during spinal surgical procedures, TES–motor-evoked potentials (TES-MEPs) are experienced as sensitive to minute impact on the spinal cord [15–18]. Indeed, TES and TMS are promising diagnostic tools for detection of subtle compromises of the myeloma in spinal cord injuries and this also accounts for horses [19]. The aim of the present review is to provide a concise overview of possible approaches for the diagnostic workup of corticospinal disorders in horses. Special attention will be paid to the utility of electrodiagnostic techniques to assess functional integrity of the corticospinal and adjunct motor tracts.

2. Neuroanatomy and Function of Spinal Motor Tracts and Neural Network

The pyramidal or corticospinal tract is the neurological tract that controls voluntary muscle contractions and modulates proprioception. The cell bodies of the upper motor neurons (UMNs) of the corticospinal tract are localized in the motor cortex of the brain. Their axons transfer efferent messages to the lower motor neurons (LMNs), of which the cell bodies are localized in the gray matter of the spinal cord [20]. The LMNs innervate muscular motor units. In humans, the descending premotor and motor pathways have monosynaptic connections to the LMNs and also produce monosynaptic excitation of motor neurons. Other messages from the cortex are mediated via extrapyramidal pathways like the rubrospinal, reticulospinal, and vestibulospinal tracts and are connected to LMNs in a multisynaptic configuration. Collaterals of the corticospinal tract communicate with extrapyramidal routes at brainstem level like connections with reticular neurons and spinal neurons at different segmental levels.

In humans and many primates, the corticospinal tract has a principal role in the control and regulation of motor activity and facilitates the specific capacity to perform skilled movements [20]. By contrast, in phylogenetic older species such as horses that split off earlier from the phylogenetic pedigree than humans, it is believed that the motor activity is likely mainly regulated via the extrapyramidal system with a subordinate contribution of the corticospinal tract. According to some anatomists, the equine pyramidal system is less well developed than the extrapyramidal system [21,22]. In fact, it seems that the pyramidal tracts in horses end most likely at the level of the midcervical region of the spinal cord from where motor neurons are activated via intersynaptic connections in which propriospinal neurons are an important intermediate station. These also receive motor input from the extrapyramidal motor tracts. The brain cortex–hind limb connections appear anatomically more complex. It is unknown to what extent experimental data of smaller animals, such as rodents, cats, rats, apply to horses. It seems obvious that these animals share large neuroanatomical and neurophysiological pathways with humans and primates.

The authors have deduced from various studies that TMS and TES stimuli likely are conducted via corticospinal in combination with extrapyramidal tracts [11,19]. However, the pathways that TES impulses follow are due to the interconnection via propriospinal interneurons more difficult to deduce in the thoracic and lumbar parts of the spinal cord than in the front legs and remain speculative. Nevertheless, the response measured in the hind legs (m. tibialis cranialis) appears not more delayed than can be explained by the putative length of the neural pathway. Of vital importance for the motor control of posture, position, and motor function of the body are connections with the proprioceptive and vestibular system including vestibular-spinal tracts, inhibiting connections from the cerebellum and sensory-proprioceptive afferents from the skin, tendons, and muscles. Whether this could result in observable modulation of TES- or TMS-evoked potentials is not known in horses.

3. Common Spinal Cord Injuries in the Horse

Damage to the spinal motor tract can be caused by many factors, leading to the expression of neurological symptoms, which can be very subtle in some cases, and thus very challenging from a diagnostic point of view. The list of differential diagnoses consists of congenital causes, such as occipitoatlantoaxial malformation, developmental disorders such as cervical vertebral stenotic myelopathy (CVSM), trauma, neurotropic infections (such as herpes myeloencephalopathy and West Nile virus), degenerative disorders (such as osteoarthrosis of mainly the caudal cervical facet joints or equine degenerative myeloencephalopathy), and neoplasia.

4. Diagnosis of Spinal Cord Injuries

Diagnosing corticospinal injuries in horses is challenging, especially in horses showing only subtle signs of spinal ataxia because the signs are often confused with signs of mild musculoskeletal lameness [23]. Therefore, in addition to complete clinical, neurological, and orthopedic examinations, clinicians sometimes need additional diagnostic techniques, such as diagnostic nerve blocks, electromyography, ultrasonography, and so on, all of which have their specific advantages and limitations. Each of these diagnostic approaches has its pro's and con's.

4.1. Clinical Examination

A thorough neurological examination is key to determine whether a neurological problem is present and to obtain a first impression of where the problem might be localized inside the patient's body. However, a clinical examination is rarely sufficient to identify the exact location of the lesion. Understanding how to use and interpret all differential diagnostic options helps the clinician to better understand the findings of the neurological examination. For example, breed, gender, and age often direct the examiner towards a particular disorder, for example, CVSM in young warmblood horses, while osteoarthrosis of the caudal articular process joints occurs in older sports horses [24,25]. Neoplastic disorders of the spinal cord (melanoma, etc.) are quite rare in horses but should be part of the differential diagnosis, especially in older horses [26]. A thorough anamnesis will inform the clinician about when the horse started to show symptoms and how these symptoms progressed over time: either static, slowly or rapidly, or recurrent on a regular basis. The behavior, position, and mentation of the horse have to be observed and the cranial nerve functions should be evaluated. Healthy horses will divide their weight equally over their four limbs. Horses with disturbed proprioception will have a basewide or more narrow stance.

Subsequently, the head, neck, and back have to be palpated and manipulated for signs of pain, bony or muscular asymmetry, focal muscle atrophy, localized sweating, and decreased pain perception. Finally, the tail tone, anal reflex, and perianal reflex should be evaluated especially when Herpesvirus myeloencephalitis is suspected.

Gait abnormalities can have a neurologic and/or orthopedic cause. Orthopedic gait abnormalities are often consistent or regularly irregular at each step, whereas neurological gait abnormalities occur irregularly and encompass different degrees of dysmetria, weakness, paresis, and/or ataxia. Based on the number of affected limbs, an anatomic localization can be designated to the pathological condition (see Table 1).

Depending on the location and seriousness of spinal cord injury, mild to severe gait deficits and loss of proprioceptive functioning can be seen [3]. It is sometimes difficult to distinguish between cerebellar ataxia (Arabian breed–cerebellar abiotrophy), vestibular ataxia, and spinal ataxia. Ataxia is considered as a lack of coordination due to lesions of either the vestibular system, or the cerebellum, or deficits at the level of the ascending sensory tracts. A widely used grading scale for ataxia is described by Mayhew et al. [2] (see Table 2). Lesions in the LMNs induce flaccid paresis, severe neurogenic muscular dystrophy, and weakened spinal reflexes, whereas lesions in the UMNs induce spasticity of the muscles and exaggerated reflexes because they cannot exert their inhibitory effect on the LMNs anymore. Several proprioceptive tests can be performed (see Table 3).

4.2. Medical Imaging and Laboratory Techniques

4.2.1. Radiography

Radiography is the most commonly used diagnostic imaging tool and is helpful to detect malalignment of the vertebrae, osteoarthrosis of the cervical articular process joints, fractures, intervertebral disk pathology, neoplasia, and so on. This technique is easy to perform on the standing sedated horse, affordable, and fast. Mean sagittal diameter of the cervical canal in horses has been determined radiographically. However, radiography is not the most ideal medical imaging technique for all anatomical locations of the corticospinal tract. High-quality and distinctive radiographs of the back are often difficult to obtain in horses because of the large body mass at that location. Radiographs of the skull can be difficult to interpret because of the complex anatomy of the skull leading to extensive superimposition of bony structures. On top of that, not all abnormalities seen on the radiographs have clinical importance [28]. On the other hand, even when the radiographs are perfectly normal, spinal cord function can be compromised. For example, cervical stenotic myelopathy can still occur and the localization of the lesion is difficult to determine based solely on standing radiographs. Lesions can also be obscured because of superimposition of the structures on radiographic images. In general, laterolateral and oblique (left lateral 45–55 ventral-dorsolateral oblique and right lateral 45–55 ventral-dorsolateral oblique) radiographic projections of the neck are recommended, especially to evaluate the articular process joints. Cervical vertebral stenotic myelopathy is characterized by flare of the caudal epiphysis of the vertebral body, malalignment between adjacent vertebrae, extension of the vertebral caudal dorsal lamina, and abnormal ossification of articular processes. Two objective ratios are established on laterolateral radiographs to assess the vertebral canal diameter: the intravertebral ratio (measured within a vertebra) and the intervertebral ratio (see Fig. 1). The intervertebral ratio takes into account the distance between adjacent vertebrae. An intravertebral ratio < 52% for C3–C6, <56% for C6–C7 [3] or intravertebral/and intervertebral ratio <48.5% for C2–C7 is indicative for Wobbler syndrome [29] (see Fig. 2). The sensitivity and specificity of the intravertebral ratio to detect pathological conditions is approximately 90% [30]. However, it must be highlighted that the spinal cord itself cannot be visualized with only radiography [31].

Table 1
Estimation of the lesion location based on manifested clinical signs [27].

Neuroanatomic localization	Predominant clinical signs
Brain–Cranial to foramen magnum	
Cerebral cortex	Postural deficits, seizures, altered mentation, blindness
Brain stem	Ataxia, paresis, dysmetria, dysphagia, anisocoria, or dilated pupils
Vestibular system	Ataxia, head tilt, pronounced postural deficits
Cerebellum	Ataxia, intention tremors
Cranial nerves	
Spinal cord	Ataxia, paresis, dysmetria, spasticity
CI–C5	All 4 limbs, worse in pelvic limbs, +/− Homer's
C6–T2	All 4 limbs, worse in thoracic limbs, +/− Horner's
T3–L3	Pelvic limbs
S3–S5	Urinary incontinence, fecal retention, hypalgesia tail, and perianal areas
Coccygeal	Decreased tail tone, hypalgesia caudal to lesion

Table 2
Grading scale for ataxia is described by Mayhew et al. [2].

Grade	Description
0	No neurologic deficits
1	Neurological deficits that are only subtly detected at normal gait, but worsen during backing, turning, loin pressure, or neck extension
2	Neurologic deficits easily detected at the walk an exaggerated by backing, turning, loin pressure, or neck extension
3	Neurologic deficits prominent at the walk combined with a tendency to buckle or fall when backing, turning, loin pressure, or neck extension; postural deficits noted at rest
4	Stumbling, tripping, and falling spontaneously at a normal gait
5	Horse recumbent

4.2.2. Myelography

Myelography is helpful to identify the location and degree of spinal cord narrowing, however, it doesn't provide conclusive information about the actual effect of the identified narrowing on functional integrity of the spinal cord (see Fig. 3). Myelography entails the slow injection of contrast fluid into the subarachnoid

Table 3
Overview of the most common proprioceptive tests performed in horses.

Proprioceptive tests	Signs of proprioceptive dysfunction
Walk slowly in a straight line	Stumbling, toe-dragging
Go backwards	Tremor
Pull the tail aside while the horse walks in a straight line	Inconsistent foot placement
Make small circles	Variable position of the limbs when the horse has to go backwards or stop
Trot in a straight line	Disunited canter
Make lots of transitions	Wide stance
Place obstacles	Delay in corrective response to abnormal positioning of the limbs
	Sway at walk
	Weak tail tone
	Hypermetria

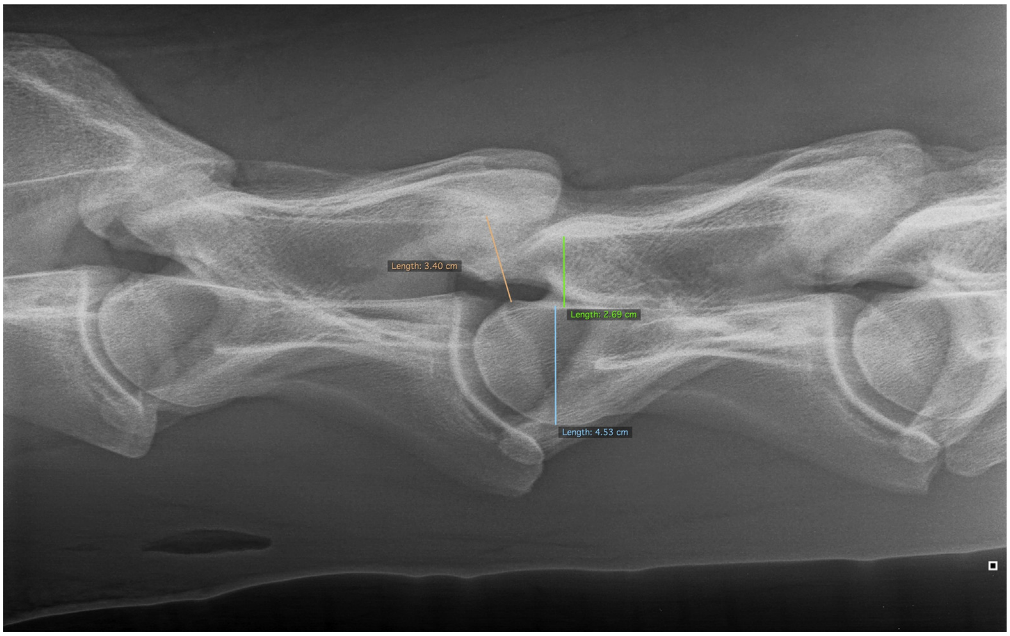

Fig. 1. Normal lateralolateral radiograph of the second to fifth cervical vertebrae (C2—C5). The intravertebral sagittal ratio is calculated as the ratio of the minimum sagittal diameter of the spinal canal (green line) to the maximum sagittal diameter of the vertebral body, taken at the cranial aspect of the vertebra and perpendicular to the spinal canal (blue line). In this case, the intravertebral sagittal ratio is 60%. The intervertebral sagittal ratio is the ratio of the minimal distance taken from the most cranial aspect of the vertebral body to the most caudal aspect of the vertebral arch of the more cranial vertebra (orange line) and the maximal sagittal diameter of the vertebral body (blue line). In this case, the intervertebral sagittal ratio is 75%.

space. The needle can be introduced between the occiput and the atlas and this should be performed under general anesthesia. Performance of this technique under sedation has been described in the standing horse but is rarely performed in practice [32,33]. Radiographs are taken with the neck in neutral, extended, and flexed positions in an attempt to assess dynamic compression of the spinal cord. Also the lumbosacral region can be injected with contrast fluid in the standing sedated horse [34]. However, performing myelography in that location can be unrewarding because in that region, often problems are encountered to obtain a good flow and distribution of the contrast fluid [33]. On top of that, the vast mass of this region hampers proper X-ray penetration, especially in adult

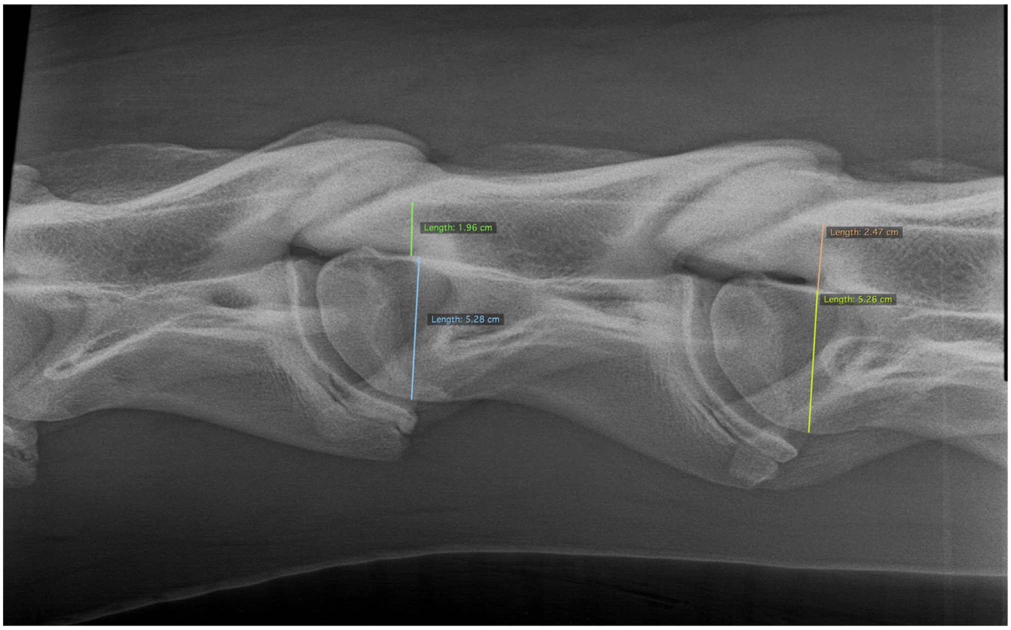

Fig. 2. Laterolateral radiograph of the third to fifth cervical vertebrae (C3—C5) of a 3-year-old stallion suffering from ataxia, hypermetry of the hind limbs, bunny hopping, and a stiff neck. Magnetic Motor-evoked potentials (MMEPs) were a abnormal in all 4 limbs. There is a moderate narrowing of the minimal sagittal diameter of the vertebral canal at the level of the fourth cervical vertebra and less pronounced at the level of the fifth cervical vertebra (intravertebral sagittal ratio of C4: 37% and C5: 47%). Note also the enlargement at the dorsal aspect of the cranial physis of the fourth and fifth cervical vertebrae and of the dorsocaudal aspects of the epiphyses of the third and fourth cervical vertebrae ("ski jumps"). There is also an asymmetry of the intervertebral disc space between C3 and C4, and to a lesser extent between C4 and C5.

Fig. 3. Myelography of an ataxic horse with cervical spinal compression (C5–C7) (Courtesy Dr Van De Winkel). Between C5–C6 and C6–C7, respectively, more than 50% decrease in sagittal diameter of the dorsal and ventral contrast columns is noticed. The sixth cervical vertebra shows a normal ossification center of the caudoventral aspect of the vertebral body.

horses but is more feasible in foals [35]. Nonetheless, the quality of radiographs of the back has greatly improved over the recent years. Moreover, it is difficult to induce a flexed position in this region to obtain dynamic views of the spinal cord [33].

Spinal cord compression is diagnosed whenever a 50% or greater decrease in the sagittal diameter of the dorsal and ventral contrast columns is seen (see Fig. 3). Possible myelography-related complications are fever, seizures, exacerbation of the neurological signs, and spinal cord trauma and could be minimalized if the puncture is performed under ultrasound guidance [33]. This technique is often combined with cerebrospinal fluid sample collection. Important to notice is that only dorsoventral and not laterolateral narrowing of the spinal canal can be identified and, of course, not all compressions identified on myelography have functional implications. Similarly, false-positive results have been reported. Van Biervliet et al. [24] suggested that it is likely that more compression (up to 70% reduction of the column) is needed to avoid false-positive results (see Table 4). Overall, myelography has been reported to have a low to moderate sensitivity and specificity [36].

4.2.3. *Cerebrospinal Fluid Analysis*

Cerebrospinal fluid (CSF) can be collected from three sites along the vertebral column: between the occiput and the atlas, between the atlas and the axis, and at the level of the lumbosacral transition. Ultrasound may be helpful to direct the needle correctly into the subarachnoid space. Lumbosacral centesis is safer than cervical centesis because at the lumbosacral location, the spinal cord terminates ahead of the lumbosacral space, the site of fluid collection [37]. Cervical centesis can be performed both under general anesthesia and in the sedated standing horse. Because of the obvious risks for general anesthesia in ataxic horses, an increasing number of clinicians perform cervical centesis with the sedated horse in standing position [38,39].

The analysis of CSF should include assessment of color, type and number of white blood cells, protein content (<80 mg/dL normal horse), and presence of red blood cells in the fluid. Xanthochromia manifests itself after intrathecal bleeding because of the production of bilirubin as a breakdown product of oxyhemoglobin [40]. Bacterial culture and PCR analysis as well as detection of antibodies can help to identify infectious or inflammatory diseases, rule out brain or spinal cord trauma, and may help to determine the time of onset of the problem.

4.2.4. *Magnetic Resonance Imaging*

Magnetic resonance imaging (MRI) can be a valuable diagnostic tool for evaluation of corticospinal lesions and provides superior soft-tissue contrast resolution and cross-sectional multiplanar projections [36,41]. Magnetic resonance imaging can be used to image vertebral column abnormalities and compression of the spinal cord and subarachnoid space. A study of Janes et al. [42] concluded that the additional visual planes and measurements, for example, vertebral canal area and the spinal cord canal area ratio, obtained by MRI are more accurate to determine the region of canal stenosis compared to radiography. The main disadvantages of MRI are the long study time, the need to put the patient under general anesthesia and its associated risks, and high costs. Moreover, up till now, MRI tubes that are big enough to scan the head together with the cranial aspect of the neck of horses are quite rare [36]. Therefore, most often, from the middle of the neck onward, it is impossible to image the neck further in caudal direction.

4.2.5. *Computed Tomography*

As an alternative to conventional radiographs, computed tomography (CT) can be performed to obtain cross-sectional and 3-dimensional images with superior anatomic detail and to better assess the osseous components of the vertebral column [43,44]. A combination of CT and myelography is an accurate method to assess minimum sagittal diameter of the spinal canal and to detect the severity and location of the narrowing and/or malformation [42]. However, again, no conclusive information with respect to

Table 4

Overview of the sensitivity and specificity of the "dorsal myelographic column (DMC) reduction rule" obtained with different cutoff values. (Compression is present when the intervertebral DMC is reduced equal to or greater than the cutoff value compared with the intravertebral DMC). More compression (65%–70% reduction of the DMC) leads to acceptable specificity.

Site	Position	75%		70%		65%		60%		55%		50%		40%		30%	
		Se	Sp	Se	Sp	Se	Sp	Se	Sp	Se	Sp	Se	Sp	Se	Sp	Se	Sp
C3 4	N	0	**100**	0	**100**	0	**100**	0	**100**	0	**97**	0	**97**	60	**90**	60	86
	F	0	89	14	89	29	85	57	81	57	78	71	78	86	74	86	67
C4 5	N	0	**100**	0	**100**	20	**97**	20	**97**	40	**97**	40	**90**	60	80	80	63
	F	60	**90**	60	**90**	60	**90**	60	83	80	80	80	73	80	63	80	60
C5 6	N	33	**100**	33	**100**	33	**97**	33	**94**	33	**90**	33	**90**	33	81	67	52
	F	0	**94**	50	**94**	50	**94**	50	**91**	50	**91**	50	88	100	85	100	73
C6 7	N	100	**93**	100	**90**	100	86	100	83	100	79	100	76	100	55	100	41
	F	50	**100**	50	**100**	50	**100**	50	**100**	50	**96**	50	**96**	50	**96**	100	81

Abbreviations: N, neutral; F, flexed.

The sensitivity (Se) and specificity (Sp) change if the cutoff values are changed.

Specificities of 90% or greater have been indicated in bold to ease the selection of decision criteria. This results in 10% or less false-positive diagnoses.

Van Biervliet et al. [24].

functional integrity of the spinal cord can be obtained. Currently, large-bore widths (85–90 cm) are becoming increasingly available to scan the head and neck up to C7 or even T2 in fully anesthetized horses [3,31,41,45]. Scans performed in lateral recumbency enable clinicians to inject contrast fluid into the spinal canal; however, once positioned in the CT scan unit, it is not possible to obtain images in flexed, nor in extended positions. Contrast CT could become the gold standard imaging modality for cervical compression myelopathy [31,46]. Computed tomography can be performed on standing sedated horses; however, in that case, the scanners can only reach until C3 in the standing position. The lack of information about the other vertebrae is an important disadvantage of CT in standing horses to diagnose spinal cord problems. However, researchers are working on purpose-designed large-bore veterinary scanners that are technically able to scan the whole spine in standing horses, though this is in its infancy [31].

4.2.6. Ultrasound

Ultrasound is a widely used noninvasive technique that is easy to perform. It does not require general anesthesia and provides a lot of useful information about the soft tissues, bones, and articulations in the neck. It is perfectly feasible to obtain dynamic images with this technique; however, it requires quite some experience because probe positioning greatly influences the obtained video clips and thus interpretation [47]. Important to keep in mind is that ultrasonography can only provide an indirect diagnosis because the visualization of the vertebral canal itself is almost impossible. This entails that diagnosis needs to be deduced indirectly based on the view of the structure of the nerve roots, surrounding soft tissue, and the articular process joints in, respectively, cervical, thoracic, and lumbar regions of the vertebral column [41]. Ultrasound can also be applied to guide CSF collection.

4.2.7. Nuclear Imaging

Scintigraphy can be used to evaluate the physiological function of tissue, but not its anatomy. The procedure entails the intravenous injection of a radioisotope and the subsequent detection of the gamma radiation by means of a gamma camera that detects so-called “hotspots” [48]. In veterinary medicine, 99 technetium hydroxymethylene-diphosphonate is used as radionuclide and allows for the assessment of bone and soft-tissue metabolic activity [49]. The soft-tissue phase is recorded 3–5 minutes after injection, while images of the bone are obtained 2 or more hours after injection [50]. The final scintigraphy images produced depend on the interaction between the tracer and the target tissue, the used radioisotope, the local blood flow, and the tissue metabolic activity. It provides information about bone remodeling, tissue metabolic activity, and ongoing pathological processes [51]. Nuclear scintigraphy is very sensitive to alterations in bone turnover [52]. This technique helps to localize possible causes of musculoskeletal pain especially for anatomic regions that are difficult to evaluate with other imaging modalities [50]. It can be helpful to evaluate presence of possible fractures or DJD-related lesions where radiography was not able to identify the lesions [31]. Owing to a poor inherent spatial resolution, anatomic detail is lost, which can be critical to differentiate physiologic from pathologic changes.

4.2.8. Epiduroscopy/Myeloscopy of the Vertebral Canal

Epiduroscopy (epidural space) and myeloscopy (subarachnoid space) encompasses the endoscopic visualization of the vertebral canal. The color, presence of swelling, and anatomy of the vertebral canal are evaluated. However, evaluation of the grade of stenosis remains subjective and challenging, especially in mild cases. These techniques are commonly performed under general anesthesia. Recently, Prange et al. [5] published a study in which lumbosacral epiduroscopy was performed in 2 standing sedated horses to avoid the risks associated with recovery after general anesthesia. Epiduroscopy seems promising because it permits the visualization of the anatomical structures within the vertebral canal up to the level of T2. The lumbosacral epidural space can be examined with myeloscopy as far cranial as L3-T18 [5]. A previous study published by Bosscher et al. [53] showed that in human patients, myeloscopy is often more successful to identify clinically relevant spinal cord abnormalities when compared to myelography [5]. Another advantage is that treatment targeting the source of the pain can be initiated immediately during performance of the endoscopy. Some authors consider epiduroscopy as a relative safe technique, associated with only mild complications (i.e., headache, pressure in the lower back, and transient paresthesia due to increase of the CSF pressure) [54], whereas other researchers report important risks such as epidural hematoma formation, trauma to the spinal cord, and accumulation of air in the epidural or subarachnoid space [6,7].

4.3. Functional Electrodiagnosis

4.3.1. Electroencephalography

Electroencephalography (EEG) is a method to record electrical activity within the brain from electrodes placed on the scalp [55]. Unfortunately, EEG records mostly spontaneous electrical activity present at the level of the superficial layers of the cerebral cortex. Therefore, this technique is useless for the detection of deep cortical lesions [56]. In veterinary medicine, EEG is sometimes used in an attempt to confirm a diagnosis of epilepsy and other brain pathologies and sometimes allows for the localization of the seizure focus [57,58].

4.3.2. Electromyography

Electromyography (EMG) can be helpful in an attempt to distinguish myogenic from neurogenic muscular disorders, to assess the integrity of motor units and to localize the lesion [59,60]. However, the degree of muscle denervation that occurs after nerve injury cannot be determined until Wallerian degeneration is complete and this can take as short as 1 week or as long as 4 weeks. Therefore, this technique ideally is not applied in an attempt to detect acute nerve injury. EMG is especially useful to diagnose “equine motor neuron disease” in horses. It is important to realize that EMG provides only information about the neuromuscular activity of a segment of the body, without providing any information concerning functional integrity of the corticospinal tract, nor the motor cortex. EMG records muscular electrical activity expressed by motor unit action potentials (MUAPs). The motor unit action potentials are recorded by needles inserted into the muscles. The activity of the muscles is registered in waveforms (MUAPs) described by amplitude, duration, and number of phases [23]. Besides MUAPs, insertional activity (when the needles are inserted into the muscles) and spontaneous activity (such as fibrillation and myotonia) are also evaluated for their presence [23]. In human medicine, EMG is reported to have a reliability between 71% and 73% [61] and a sensitivity of ±80% to seperate neurogenic and myopathic disorders [62]. EMG has been used in veterinary medicine for many years to distinguish between a healthy and neurologically abnormal horse; however, pinpointing location and type of lesion in patients remains very challenging. A study of Williams et al. [63] suggests that EMG has more value as a comparative tool within individual horses than across different horses due to poor reproducibility of the technique.

4.4. Transcranial Brain Stimulation

In horses, TMS and multipulse TES are interchangeable transcranial stimulation techniques suitable to assess motor function of the spinal cord. Elicited muscle motor-evoked potentials (MEPs) reflect the functional properties of neural elements of the route along brainstem nuclei, extrapyramidal motor tracts, propriospinal neurons, and motor neurons.

4.4.1. *Transcranial Magnetic Stimulation*

Transcranial magnetic stimulation is a noninvasive, sensitive, and painless diagnostic test to assess functional integrity of the corticospinal nervous system in horses. It is a method of brain stimulation by means of a coil that is placed on the forehead of a standing sedated horse and that generates magnetic fields passing through the motor cortex (Fig. 4). The stimulation pulses pass through the coil with varying intensities and in single or multiple pulse trains (mostly monophasic or sometimes biphasic pulses) and induce an excitatory or inhibitory activity of the neurons depending on the chosen parameters [64]. This induces electrical currents parallel to the windings of the coil that depolarize axons in the motor cortex. After motor cortex stimulation, the generated action potentials are relayed by the UMNs before further conduction takes place to the LMNs, which eventually will activate the contralateral motor unit [65]. The created magnetic motor-evoked potentials (MMEPs) can be recorded with electrodes into the contralateral muscles (m. extensor carpi radialis and m. tibialis cranialis) by means of an EMG. However, other muscles can be used to localize the problem more precisely at segmental nerve root levels. Recent studies have demonstrated, at least in animal models, that both pyramidal and extrapyramidal tracts seem to be involved in TMS and TES [11,65,66]. As mentioned previously, the cortex—hind limb connections appear anatomically more complex when compared to the cortex-forelimb connections. It is difficult to explain how exactly the extrapyramidal system with its inhibiting properties on interneurons eventually can cause an observable MEP of a flexor muscle of the tarsus.

The coil can have either a round or figure-of-eight shape. For more focal stimulation, the latter is preferred because the currents summate at the center of the “8” [64,67]. However, in horses, the round coil is still preferred because it is expected to increase the effectiveness and depth of stimulation [68] (see Fig. 4). The limiting factor of TMS is the depth at which stimulation penetrates the brain because the generated electromagnetic fields attenuate with increasing distance from the coil. This implicates that TMS only stimulates the superficial layers of the head, namely the scalp, the skull, and the meninges, without activation of the nociceptors, which explains why this technique is painless [68]. Hence, the amplitude of the magnetic field has to be high enough to generate electrical currents with sufficient energy to depolarize the corticospinal tract resulting in the production of a MMEP [69]. The evaluation of the integrity of the descending corticospinal tract is performed by assessing a set of MMEP wave parameters such as latency time, peak-to-peak amplitude, and shape [70,71]. The latency time is a measure for the conduction speed of action potentials along connecting axons. It depends on the myelinization and size of the conducting axons and the axonal length between stimulation and recording sites. The latency time is the time interval between the delivered stimulus on the forehead of the horse and onset of the resulting muscular response [65]. The peak-to-peak amplitude of the MMEPs is a measure of the amount of recruited LMNs [71]. Peak-to-peak amplitude is measured from the minimum of the most negative to the maximum of the most positive peak [65]. However, these amplitude values have to be carefully interpreted because a high interindividual/intraindividual variation has been described [72,73]. TMS is a possible addition for noninvasive imaging of the equine nervous system to better localize the compressive lesion objectively. Compression of the spinal cord can be detected by an increased motor latency time and decreased muscular MEP amplitudes.

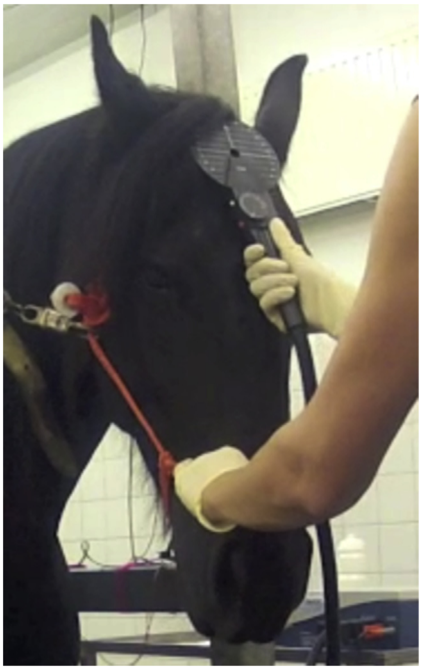

Fig. 4. Transcranial magnetic stimulation in a horse.

The downside of this technique includes important interobserver and intraobserver variability of repeated measures, especially in peak-to-peak amplitude recordings [73]. The position of the coil, variation in needle placement between patients, differences in response quality between horses, and thus, most importantly, issues with reproducibility tend to make TMS less reliable.

4.4.2. *Transcranial Electrical Stimulation*

4.4.2.1. Mechanisms of Action. Transcranial electrical stimulation (TES) was first discovered in 1980 by Merton and Morton and is an attractive alternative for TMS. Transcranial electrical stimulation modulates the resting membrane potential by applying an electrical impulse through two subcutaneously inserted electrodes on the forehead of the horse (a cathode and an anode) (see Fig. 5). Anodal polarization induces depolarization of the membranes and thus increases excitability of vertically oriented axons of upper motor neurons, while cathodal polarization induces a hyperpolarization and thus decreases their excitability [9].

Electrical stimulation activates mainly the corticospinal fibers directly due to a bypass of the motor cortex and upper motor neurons, which means that the muscular responses are less sensitive to the cortical activity level [13]. The TES-activated sites are located deeper at subcortical locations in the brain when compared to TMS and may most likely reach the cerebral peduncle and the pyramids (where the pyramidal tracts decussate) [74].

The size of the transcranial stimulation current thresholds depends on the location of the electrodes, the stimulation polarity, pulse widths, and interpulse intervals when multipulse stimulation is used [75]. Compared to direct cortical stimulation, 25% of the applied TES current arrives in the cortex [76].

4.4.2.2. Assessment of Muscular Motor-Evoked Potentials. After brain stimulation, a MEP is created and can be recorded in the muscles contralateral to the polarized brain. MEPs are recorded from subcutaneously placed needle electrodes over the musculus extensor carpi radialis (ECR) and the musculus tibialis cranialis (TC)

Fig. 5. A duo of subcutaneously inserted electrodes used with the TES technique. TES, transcranial electrical stimulation.

(see Fig. 6A–C). The key parameters of the applied electrical stimulus are current intensity (expressed in milli Ampere or Voltage), pulse duration (expressed in micro seconds), and the interpulse interval (expressed in milli seconds). Multiplication of current intensity, duration of the pulse, and number of pulses in a pulse-train determines the final magnitude of the applied stimulus (expressed in microcoulombs) [77]. The resulting MEPs are characterized by their amplitude, latency time, and shape.

To create a "normal" MEP, presence of intact motor units including alpha motor neurons, intact neuromuscular junctions, and innervated muscle fibers is mandatory. In Figure 7, a normal TES-induced MEP is depicted, providing a view on all three MEP characteristics: amplitude, latency time, and shape. The amplitude of the MEP is also influenced by the excitability of alpha motor neurons, which varies individually and over time due to changing systemic conditions and mostly unknown modulating factors [78]. The interpretation of MEPs is complicated due to varying wave shapes and amplitudes. Figure 8 shows abnormal MEPs with significant increased latency times, long polyphasic waveforms and baseline EMG activity of the left and right extensor carpi radialis. The spontaneous EMG activity is already present in the ECR before the MEP actually appears. The absence of MEPs suggests presence of a defective motor function (see Fig. 8). Obviously, an increase in latency times in all muscles and MEP responses with marked decreased amplitudes (early transcranial part of the response) likely refers to a conduction pathology in the spinal cord when peripheral nerve conduction abnormalities can be excluded. A decrease in amplitude or increase in threshold may indicate a disturbed spinal cord or brain function. Sometimes, a "silent period" can be observed after each MEP. This silent period increases in duration under normal conditions linearly with increasing stimulus intensities [77,79].

4.4.2.3. Safety Issues. Transcranial electrical stimulation is painless and usually well tolerated in horses [10,11,19]. Still, horses need to be sedated for safety reasons. In human studies, scalp burns have been reported after excessive transcranial electrical stimulation as applied during electroconvulsive therapies. Another reported complication in human medicine is the risk for seizuring after TES stimulation and the occurrence of bite injuries with an estimated incidence of 0.2% [13,80,81]. The latter are caused by jaw muscle contractions likely mediated through trigeminal nerve stimulation. None of the aforementioned injuries have been reported for TES in horses [10].

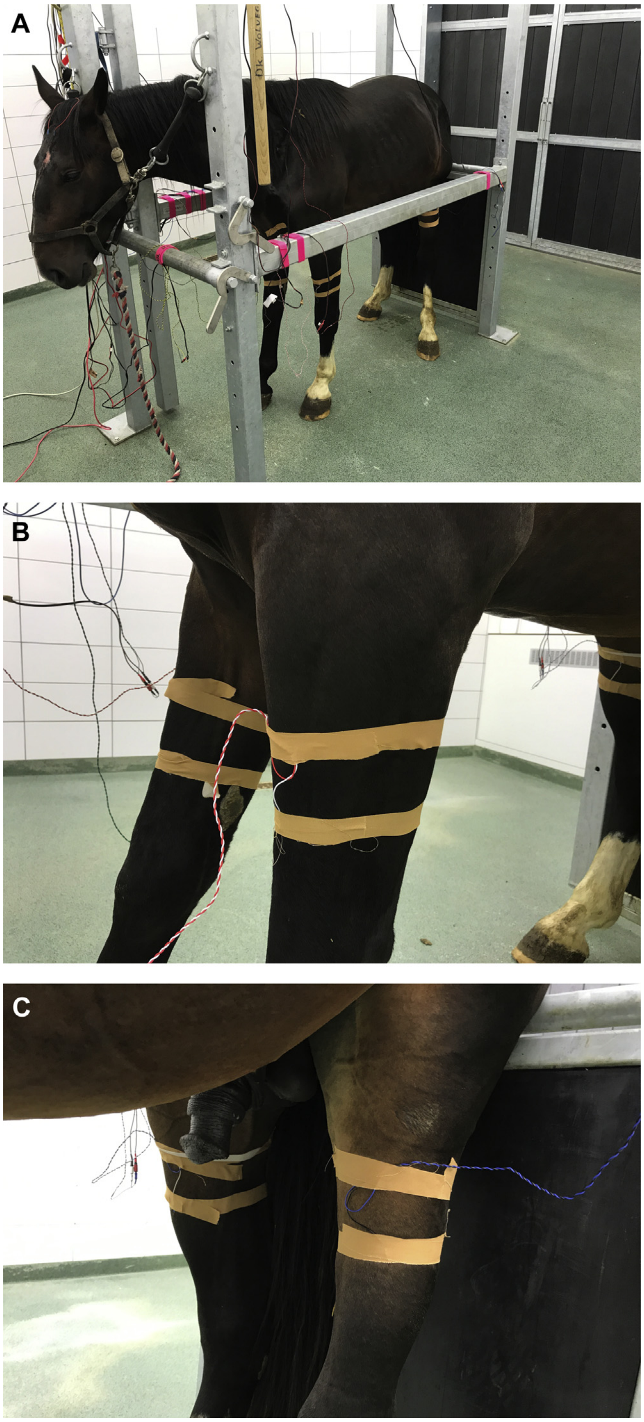

Fig. 6. (A–C) Placement of the EMG needles to measure muscular activity (m. extensor carpi radialis and m. tibialis cranialis).

4.4.2.4. Comparison of TES and TMS. As mentioned previously, both TMS and TES are used to evaluate the functional integrity of the corticospinal and associated motor tracts. Both techniques seem also promising for diagnosing Wobbler syndrome because they show lesions exclusively in the motor tract. Histopathology by

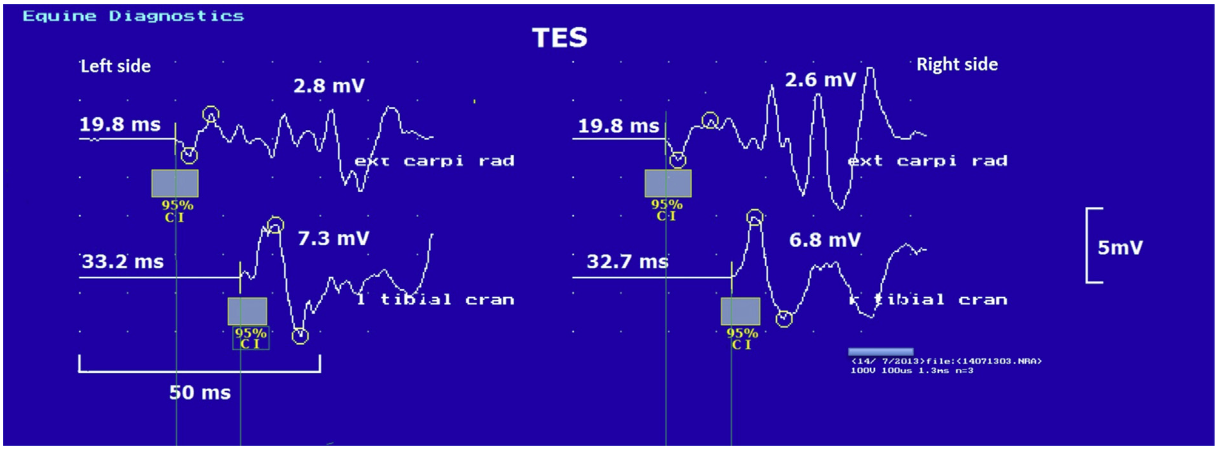

Fig. 7. Normal electrical motor-evoked potentials recorded from the extensor carpi radialis and tibialis cranialis muscles induced by TES in a horse. TES, transcranial electrical stimulation.

Yovich et al. [82] showed that in Wobbler syndrome, myelin degeneration or loss at the level of the compressive lesion was greatest in the ventral and lateral funiculi and less consistently present in the dorsal funiculi. Therefore, it can be expected that the lateral corticospinal tract and rubrospinal tracts are damaged in Wobbler syndrome. This damage should be picked up using TES and TMS techniques.

Transcranial electrical stimulation has only been validated recently for application in horses and proved to be a very promising alternative diagnostic technique besides TMS. Normative values of motor latency times (MLTs) are for TES: 16–22.6 ms (mean ± 2SD) for the ECR and 31.5–41.1 ms for the TC muscles. Based on normative values published in literature on TMS, no significant difference between both techniques can be claimed at this point. However, pairwise comparison of MLTs of both techniques in horses with evident neurological motor symptoms shows markedly increased latency times in TMS when compared to TES [11]. Table 5 shows an overview of the mean latency times and amplitudes recorded in normal healthy horses by means of TMS and TES. Currently, most TMS tests use intramuscular needle electrodes, which are more difficult to place and do not stay in place as good as surface electrodes [71,85]. A more recent study investigated the use of surface electrodes in TMS [73]. In spite of the study limitations, these electrode types seem to offer an alternative. More research is needed with that respect. Transcranial electrical stimulation tests are currently performed with subcutaneous needle electrodes [11]. Because of the fixed position of the stimulation electrodes, no coil repositioning errors have to be taken into account with TES. The latter can be challenging with respect to reproducibility of the TMS technique when repetitive placing of the magnetic TMS coil over the dome-shaped forehead of the horse is needed. A good reproducibility of repeated measurements has been reported within the same horse for TES. Moreover, the direction of current flow is different for the two brain stimulation techniques [64]. This implies that the radially oriented corticospinal axons will have a higher activation threshold for the TMS than for the TES technique and that only a small fraction of the electrical currents will actually flow through the brain when TMS is applied [74]. Furthermore, the selectivity of very small areas of the brain is easier with TES because the spatial resolution of TMS is between 0.5 and 1 cm [67].

Transcranial electrical stimulation produces D-waves (direct waves) mainly, by direct stimulation of motor axons of pyramidal neurons, but activates several other cortical synaptic circuits also indirectly (I-waves). Transcranial magnetic stimulation produces mainly I-waves because first cortical interneurons are stimulated, before the corticospinal tract is aroused. These D-waves and I-

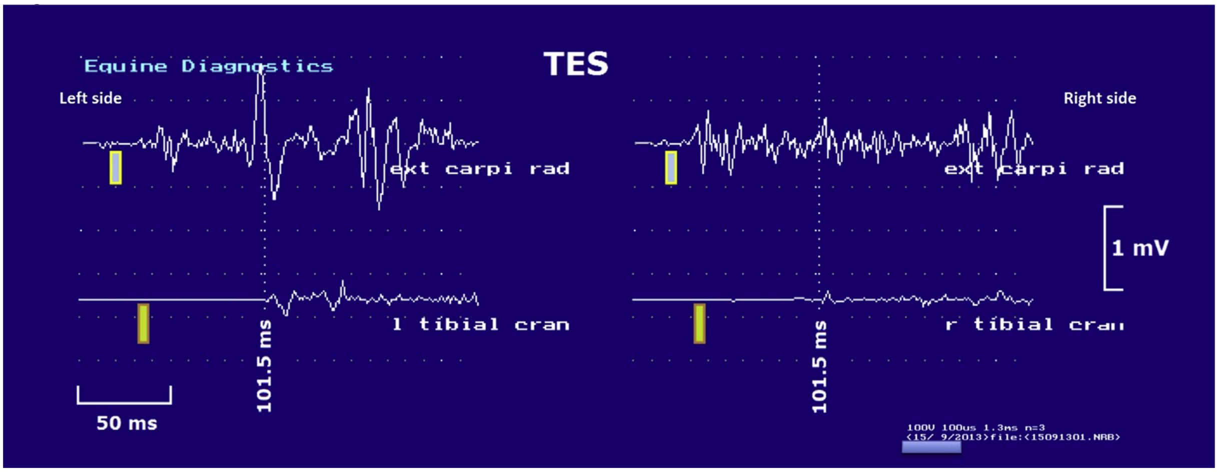

Fig. 8. Pathological electrical motor-evoked potentials of the extensor carpi radialis and tibialis cranialis muscles induced by TES in a horse with cervical stenotic myelopathy. TES, transcranial electrical stimulation.

Table 5
Overview of the mean latency times and amplitudes recorded in healthy horses reported for TMS and TES.

Curve parameters	TMS	Remarks	Source	TES	Remarks	Source
MLTs thoracic limb	20.8 ± 1.5 ms	IM needle electrodes	[73]	20.8 ± 1.85 ms (left) 19.7 ± 1.69 ms (right)	Subcutaneous needle electrodes 12 horses	[83]
	21.2 ± 1.4 ms	Surface electrodes	[73]	18.6 ± 1.26 (left) 18.4 ± 1.1 (right)	SC needle electrodes 5 horses	[10]
	19.0 ± 2.3 ms	Surface electrodes 10 ponies	[70]	19.7 ± 1.48 (left) 19.1 ± 0.83 (right)	12 horses	[84]
	20.81 ± 1.85 ms (left) 20.59 ± 1.83 ms(right)	IM needle electrodes 12 horses	[71]			
	19.32 ± 2.5 ms	84 horses	[85]			
MLTs pelvic limb	39.4 ± 3.8 ms	IM needle electrodes	[73]	34.6 ± 2.01 ms (left) 34.9 ± 1.69 ms (right)	Subcutaneous needle electrodes 12 horses	[83]
	39.2 ± 3.8 ms	Surface electrodes	[73]	34.5 ± 0.96 (left) 33.4 ± 1.52 (right)	SC needle electrodes 5 horses	[10]
	30.2 ± 3.4 ms	Surface electrodes 10 ponies	[70]	36.17 ± 2.12 ms (left) 36.32 ± 2.4 ms (right)	SC needle electrodes 12 horses	[84]
	35.94 ± 3.43 ms (left) 36.33 ± 3.53 ms (right)	IM needle electrodes 12 horses	[71]			
	30.54 ± 5.28 ms	84 horses	[85]			
Mean amplitude thoracic limb	8.3 ± 4.1 mV	IM needle electrode	[73]	3.61 ± 2.55 mV (left) 4.53 ± 3.1 mV (right)	SC needle electrodes 12 horses	[84]
	7.2 ± 4.7 mV	Surface electrodes	[73]			
	7.37 ± 2.69 mV (left) 7.62 ± 2.68 mV (right)	IM needle electrodes 12 horses	[71]			
	9.54 ± 3.73 mV	IM needle electrodes 84 horses	[85]			
Mean amplitude pelvic limb	4.2 ± 3.1 mV	IM needle electrode	[73]	2.66 ± 2.22 mV (left) 2.55 ± 1.85 mV (right)	SC needle electrodes 12 horses	[84]
	3.8 ± 2.4 mV	Surface electrodes	[73]			
	5.02 ± 3.87 mV (left) 4.26 ± 2.55 mV (right)	IM needle electrodes 12 horses	[71]			
	6.62 ± 3.62 mV	IM needle electrodes 84 horses	[85]			

Abbreviations: TES, transcranial electrical stimulation; MLTs, motor latency times.

waves can be recorded with electrodes positioned into the epidural space, while the muscular MEPs from neighboring muscles or M-waves are recorded by subcutaneous needle electrodes positioned into muscles [74]. Figure 9 shows the D-, I-, and M-waves. Compared to TMS, motor latency time is significantly shorter in TES due to the direct stimulation [12]. The latency time in horses seems

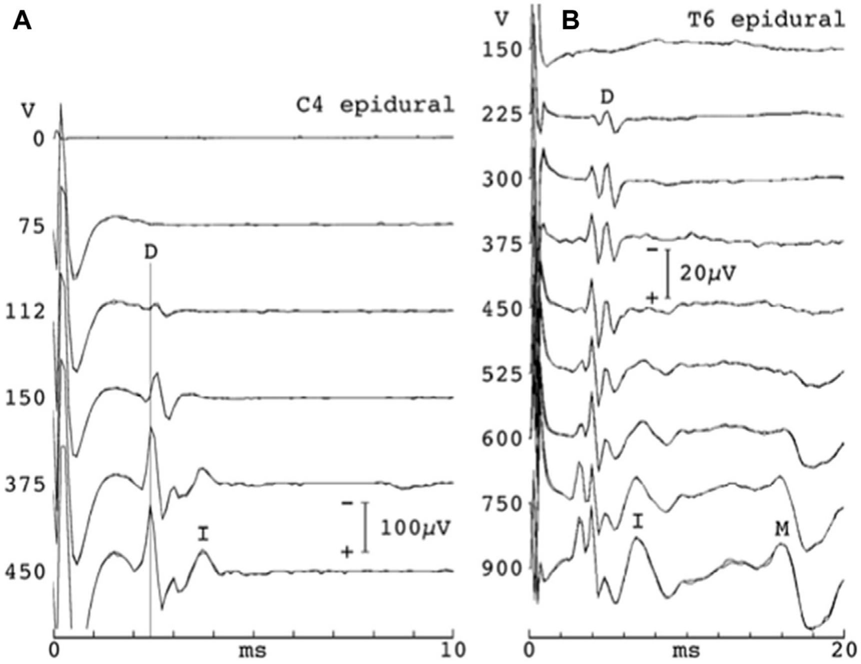

Fig. 9. Spinal epidural responses with increasing voltages (V) in anesthetized human patients. With increasing voltages, the D-wave amplitudes increase and the latency times decrease. At higher intensities, I-waves are also recruited, and at very high intensity, M-waves also appear [13].

to be a valuable tool in TMS and TES to detect small pathological changes in the descending corticospinal tract [10,73]. Journée et al. [10] showed a very small standard deviation in latency times between horses with the same height.

Direct stimulation of the corticospinal tract enhances the sensitivity of TES and renders this technique less sensitive to cortical function or sedative effects. For this reason, TES is a reliable technique to functionally assess corticospinal integrity [84]. When compared to TES, the differences in neural stimulation sites may be reflected by extra prolonged TMS latency times in horses with evident neurological motor symptoms [11].

Both TMS and TES are widely used as diagnostic techniques in humans; however, they can also be used therapeutically. However, the exact way in which repetitive TMS and TES induce long-lasting electrophysiological effects on the motor cortex is not yet fully understood.

5. Conclusions

Spinal cord disorders are often encountered in horses, but defining the exact localization and cause of the lesions and the evaluation of the functional damage of the spinal cord is still a challenge for equine clinicians. The most commonly used diagnostic approach starts with a neurological clinical examination, combined with vertebral radiographs. Radiographs, ultrasonography, and scintigraphy have limited ability to accurately identify abnormalities. Superposition of osseous and soft-tissue structures and the wide range of radiographic findings in healthy horses make identification of clinically significant findings difficult. Myelography and cerebrospinal fluid analysis are useful but more invasive techniques. Magnetic resonance imaging and especially CT are due to recent advances in bore width combined with contrast the most promising imaging techniques to diagnose the underlying causes of spinal cord disease. However, none of the aforementioned diagnostic approaches allow for evaluation of the functional integrity of the spinal cord, despite presence of identified narrowing. Brain stimulation diagnostics have emerged as promising diagnostic tools for that purpose. Besides TMS, TES is a sensitive technique to assess spinal motor function. Recent research shows that TES is highly reproducible, and easily applicable.

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