FACULTY OF VETERINARY MEDICINE
accredited by EAEVE

Magnetic motor evoked potentials:

A diagnostic test for spinal cord dysfunction

in large animals

Joke Rijckaert

Dissertation submitted in fulfilment of the requirements

for the degree of Doctor of Veterinary Science (PhD)

2019

Promotoren:

Dr. B. Pardon

Prof. dr. L. Van Ham

Prof. dr. G. van Loon

Department of Large Animal Internal Medicine

Faculty of Veterinary Medicine

Ghent University

GHENT UNIVERSITY

Magnetic motor evoked potentials: a diagnostic test for spinal cord dysfunction in large animals

Magnetisch uitgelokte motorische potentialen: een diagnostische test voor ruggenmerg dysfunctie bij grote huisdieren

Rijckaert Joke

Vakgroep Inwendige Ziekten van de Grote Huisdieren,

Faculteit Diergeneeskunde, Universiteit Gent,

Salisburylaan 133, 9820 Merelbeke, Belgium

MEMBERS OF THE JURY

Prof. dr. G. van Loon, *promotor*
Department of Large Animal Internal Medicine, Faculty of Veterinary Medicine, Merelbeke, Belgium

Dr. B. Pardon, *promotor*
Department of Large Animal Internal Medicine, Faculty of Veterinary Medicine, Merelbeke, Belgium

Prof. dr. L. Van Ham, *promotor*
Small Animal Department, Faculty of Veterinary Medicine, Merelbeke, Belgium

Prof. dr. J. Dewulf, *head of the jury*
Department of Obstetrics, reproduction and herd health, Faculty of Veterinary Medicine, Merelbeke, Belgium

Prof. dr. L. Göhring,
Ludwig-Maximilians-Universität München, Klinik für Pferde, Veterinärstr. 13, D-80539 München, Duitsland

Dr. A. Viaene,
Department of Physical Medicine and Rehabilitation, Revalidation service, University hospital, Ghent

Dr. M. Dumoulin,
Department of surgery and anesthesiology of domestic animals, Faculty of Veterinary Medicine, Merelbeke, Belgium

Dr. S. Bhatti, *Secretary*
Small Animal Department, Faculty of Veterinary Medicine, Merelbeke, Belgium

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

AMPL	Amplitude
AMPL N	Needle recording amplitude
AMPL S	Surface recording amplitude
C1-7	Cervical vertebra 1-7
CI	Confidence interval
cm	Centimeter
CMCT	Central motor conduction time
Cn	Cervical nerve
covDn	Covariance for negatives
covDp	Covariance for positives
CSF	Cerebrospinal fluid
CT	Computed tomography
CUSUM	Cumulative sum control
CV	Coefficient of variation
CVCM	Cervical vertebral compressive myelopathy
CVM	Cervical vertebral malformation
CVSM	Cervical vertebral stenotic myelopathy
e.m.	Extra muscular
EC	Extensor carpi radialis muscle
ECG	Electrocardiogram
ECR	Extensor carpi radialis
EDM	Equine degenerative myelopathy
EMG	Electromyography
EPM	Equine protozoal myeloencephalitis
HE	Hematoxylin and eosin
i.m.	Intramuscular
kg	Kilogram

kV	Kilovolt
kW	Kilowatt
L1-5	Lumbar vertebra 1-5
LAT	Latency time
LAT N	Needle recording latency time
LAT S	Surface recording latency time
mAs	Milliampere-seconds
Max	Maximum
Min	Minimum
mm	Millimeter
MMEP	Magnetic motor evoked potential
MRI	Magnetic resonance imaging
ms	Milliseconds
mV	Millivolt
n	Number of cases
NAD	Neuroaxonal degeneration
NP	Not performed
OC	Osteochondrosis
RI	Reference interval
ROC	Receiver operating curve
s.c.	Subcutaneous
SD	Standard deviation
sEMG	Surface EMG
Se_{NeurEx}	Sensitivity of Neurological examination
Se_{RX}	Sensitivity of cervical radiographs
Se_{TMS}	Sensitivity of Transcranial magnetic stimulation
$Sp_{aNeurEx}$	Specificity of Neurological examination
Sp_{RX}	Specificity of cervical radiographs
Sp_{TMS}	Specificity of Transcranial magnetic stimulation
T	Tesla

T1-T9	Thoracic vertebra 1-8
TC	Tibialis cranialis muscle
TES	Transcranial electrical stimulation
TMS	Transcranial magnetic stimulation

Spinal ataxia, caused by spinal cord disease, is a common neurological disease in horses and cattle. Clinical signs range from subtle to very obvious with recumbency in the worst case. Also in recumbent cattle, neurological disease is an important differential diagnosis. Because of the size of these large animals, recumbency is life-threatening. However, in the case of horses, also the most subtle signs have an important impact on a horses' life, as for (optimal) jumping, dressage, eventing, racing,… performance, a perfect gait is required. Because poor performance of equine athletes will cost owners and trainers a lot of time and money, a reliable diagnosis, also in mild cases, is absolutely necessary.

Mostly, diagnosis of spinal cord disease is based on a combination of patient history, neurological examination and diagnostic imaging, complemented with blood and cerebrospinal fluid analysis, whenever necessary. However, sensitivity and specificity of the different tests are rather low and they do not give information about the function of the spinal cord. Therefore, transcranial magnetic stimulation with recording of magnetic motor evoked potentials (TMS-MMEP) has been suggested as an alternative. However, the technique of TMS-MMEP can still be improved, more precise lesion localisation is wanted and as there is no gold standard for diagnosis of spinal cord disease in horses, the accuracy of the diagnostic test was still unrevealed. Therefore, in this thesis, answers on these shortcomings were provided and the value of TMS-MMEP, in reference to clinical examination and radiography, was demonstrated.

CHAPTER 1

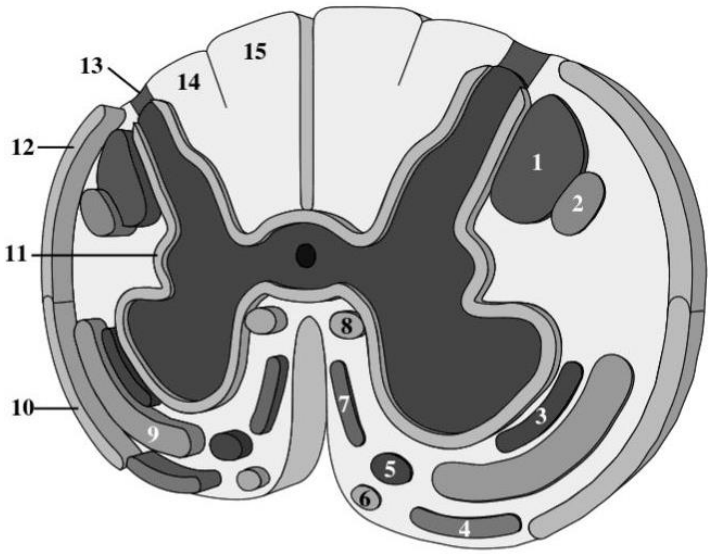
GENERAL INTRODUCTION

SPINAL CORD DYSFUNCTION AND ATAXIA IN LARGE ANIMALS

The term "ataxia" is derived from the Greek word, "a taxis" which means "without order". Horses with ataxia show incoordination and are unable to control their movements because of neurological disease. Depending on the localization of the causative lesion, there are 3 different types of ataxia in large animals, cerebellar, vestibular and spinal ataxia, each presenting different neurological signs (Alcott, 2017). Cerebellar ataxia is rare and may be developmental or acquired from structural of inflammatory disturbances. Typical clinical signs are intention tremors of the head and the absence of the menace response. Vestibular ataxia results from lesions in the peripheral (inner ear and peripheral part of the vestibular nerve) or central (nuclei of the vestibular nerve in the brainstem) vestibular system. In the most typical presentation, horses show head tilt, pathological nystagmus, leaning and circling to the affected side. The last type, spinal ataxia, is the most common form and is also called general proprioceptive or sensory ataxia. These horses show no abnormalities at the level of the head but move uncoordinated because of spinal cord dysfunction.

The spinal cord is the connection between the brain and the peripheral spinal nerves which is necessary for transmission of sensory and motoric information. The afferent and efferent pathways for this transmission are situated at the outside and form the white matter of the spinal cord. The sensory pathways are mainly located dorsal and lateral while the motoric tracts are situated more ventral and medial. Centrally in the spinal cord, the butterfly shaped grey matter, containing the neural cell bodies, is found (Mayhew, 2008).

For a normal gait, both sensory and motoric pathways need to function normally. The sensory tracts are necessary for general proprioception. This detection of the position and the movement of muscles and tendons is achieved by 2 different pathways: 1 pathway for transmitting unconscious information to the cerebellum and 1 for conscious proprioceptive information to the cerebral cortex. The first pathway uses the dorsal and ventral spinocerebellar pathway situated lateral in the spinal cord. The second uses the fasciculus gracilis and the fasciculus cuneatus, located dorsally in the spinal cord (figure 1) (De Lahunta and Glass, 2009).



1. ***Lateral corticospinal tract***
2. **Rubrospinal tract**
3. **Medullary reticulospinal tract**
4. Lateral vestibulospinal tract
5. **Pontine reticulospinal tract**
6. Tectospinal tract
7. ***Ventral corticospinal tract***
8. Medial longitudinal fasciculus
9. Spinothalamic tract
10. **Ventral spinocerebellar tract**
11. Fasciculus proprius
12. **Dorsal spinocerebellar tract**
13. Dorsolateral fasciculus
14. **Fasciculus cuneatus**
15. **Fasciculus gracilis**

Figure 1. Position of ascending and descending tracts in the spinal cord. Descending tracts are numbered on the right side and ascending tracts on the left (from Furr and Reed, 2008).

The motoric pathways are necessary for initiation of voluntary movement, muscle tone and posture. The motoric pathways are a part of the upper motor neuron system which regulates the lower motor neurons, the peripheral neurons who directly innervate the muscles. The motoric system is divided into a pyramidal and extrapyramidal component. The pyramidal system determines the capacity to perform skilled movements and is of minor importance in horses. The extrapyramidal system is of much greater importance as it is responsible for the regulation of stereotyped motor activities. Pyramidal efferent information is transmitted through the corticospinal tract, extrapyramidal information through the rubrospinal and the pontine and medullary reticulospinal tracts (Furr and Reed, 2008).

Any lesion affecting the sensory pathways will cause general proprioceptive ataxia, but mostly also the motoric pathways are disturbed (Alcott, 2017). At the site of the lesion, both ascending and descending tracts will degenerate, but because of progressive Wallerian degeneration also the afferent tracts cranial and the efferent tracts caudal of the lesion will be affected (figure 2).

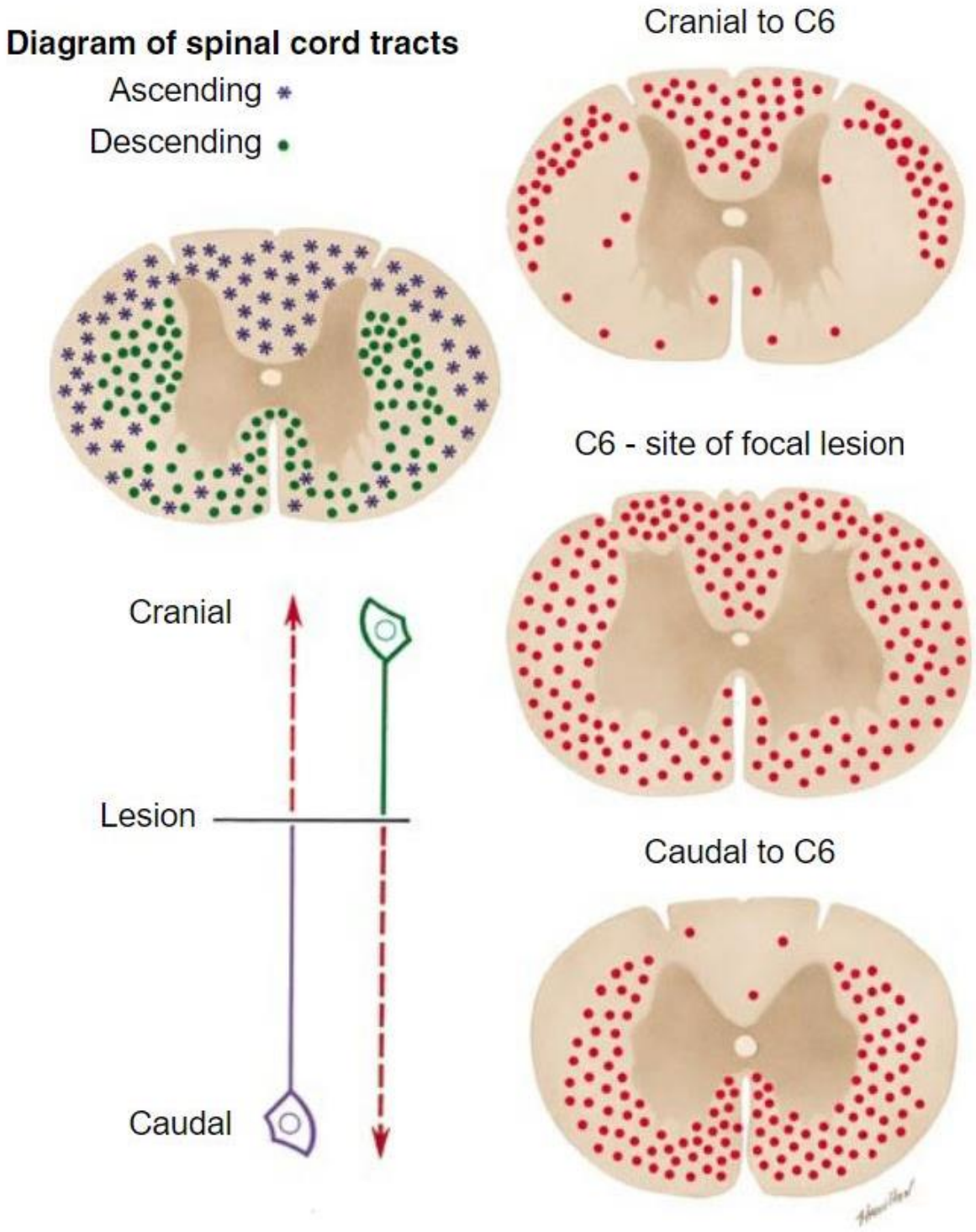


Figure 2. Pattern of spinal cord degeneration following a focal segmental lesion at the level of C6. Cranial to C6 wallerian degeneration affects the afferent tracts, located dorsal and lateral in the spinal cord. Caudal to C6 the efferent tracts, located more medial and ventral, show degeneration (from De Lahunta and Glass, 2009).

Depending on the location of the lesion, upper or lower motor neuron symptoms may occur. Clinical signs of upper motor neuron disease are paresis caused by a delay in limb protraction, spasticity, hyperreflexia and release from inhibition. With lower motor neuron disease also paresis can be seen or even complete paralysis, hypo- or areflexia, hypo- or atonia and muscle atrophy. The horse might be unable to support its weight. Lumbosacral lesions cause lower motor neuron signs and more cranially located lesions cause upper motor neuron signs in the pelvic limbs. In case of a caudocervical lesion, additionally lower motor neuron signs are present. If the lesion is located in the craniocervical spinal cord, upper motor neuron signs occur in pelvic and thoracic limbs (De Lahunta and Glass, 2009).

Lesions causing proprioceptive ataxia can be categorised as compressive, inflammatory or degenerative (table 1) (Alcott, 2017). In North American horses, the most common causes of spinal ataxia are the inflammatory equine protozoal myeloencephalitis (EPM) and equine degenerative myelopathy (EDM)/neuroaxonal degeneration (NAD) (Hamir et al., 1992; Mayhew et al., 1978) but, in the rest of the world the most important cause of ataxia in sport horses is spinal cord compression caused by cervical vertebral malformation (CVM) (Nappert et al., 1989; Oswald et al., 2010).

Table 1. Differential diagnosis for spinal ataxia in horses, most common causes

Compression on the spinal cord	• Cervical vertebral stenotic myelopathy • Trauma (vertebral fractures, oedema, hematoma, luxations…) • Neoplasia • Occipito atlanto axial malformation • Intervertebral disk disease • Abscess
Degenerative lesions	• Secondary to spinal cord compression • Equine degenerative myeloencephalopathy (EDM) • Neuroaxonal dystrophy (NAD)
Inflammatory lesions	• Equine herpesvirus myeloencephalopathy • Osteomyelitis • Equine protozoal myeloencephalitis • Abscess

CERVICAL VERTEBRAL MALFORMATION

DEFINITION

Cervical vertebral malformation, cervical vertebral malarticulation, cervical vertebral stenotic myelopathy (CVSM), cervical spondylotic myelopathy, wobbler: all synonyms for the same disease referring to several possible malformations of the cervical vertebrae in horses. These malformations cause compression of the spinal cord and therefore an abnormal, uncoordinated, wobbly gait and weakness in horses. The ataxia will be more severe in the pelvic limbs, and less obvious in the thoracic limbs, because of the more superficial location of the proprioceptive tracts to the pelvic limbs (Nout and Reed, 2003). The severity of clinical ataxia can be graded from 0-5 (Table 1) (Mayhew et al., 1978).

Table 2. Grading system for ataxia (Mayhew et al., 1978)

Grade 0:	Normal strength and coordination
Grade 1:	Subtle neurological deficits only noted under special circumstances but mild
Grade 2:	Mild neurological deficits but apparent at all times/gaits
Grade 3:	Moderate deficits at all times/gaits that are obvious to all observers
Grade 4:	Severe deficits with tendency to buckle, stumble spontaneously, and trip and fall.
Grade 5:	Recumbency

There are 2 types of cervical vertebral malformation: a dynamic and a static type. The dynamic form is typically seen in very young horses and results from a subluxation and instability of the cervical vertebrae causing spinal cord compression at the level of C3-C4. The static type, is found in older horses, typically between 2 and 5 years of age. The spinal cord compression is located in the caudal part (C5-C7) of the neck and is permanent. The causes can be bony malformations (osteoarthrosis), cartilage abnormalities or swelling of dorsal longitudinal ligaments (Mayhew et al., 1978).

PREVALENCE AND EPIDEMIOLOGY

Limited data are available about the general prevalence of CVM but, in thoroughbred horses, the prevalence is estimated at about 1.3-2.0% (Oswald et al., 2010). CVM is certainly one of the most common causes of neurologic disease in sport horses (20-30% of the neurological abnormal horses are diagnosed with CVM) (Hamir et al., 1992; Nappert et al., 1989; Tyler et al., 1993) and it is the number one cause of ataxia (up to 58% of ataxic horses are diagnosed as CVM) (Nappert et al., 1989).

CVM is more frequently seen in male horses than in mares (Levine et al., 2008; Levine et al., 2010). Thoroughbreds, Warmbloods and Tennessee Walking Horses are predisposed (Levine et al., 2008; Levine et al., 2010; Szklarz et al., 2018). According to Levine et al. (2008) and Levine et al. (2010) the diagnosis is mostly made in young horses (<4 years old, with a peak in 1-2 year olds), but CVM is also common in older age categories (Levine et al., 2007). An association with weight is not definitely confirmed, because of confounding factors as age and breed (Levine et al., 2008; Szklarz et al., 2018). In general rapidly growing horses are at a higher risk to develop the disease (Nout and Reed, 2003). The high growth rate is evidently correlated with daily management factors, which might be influencing factors.

ETIOLOGY AND PATHOGENESIS

The pathogenesis of CVM remains unclear, but is thought to be a multifactorial, developmental abnormality, modulated by genetic predisposition and environmental influences. A genetic predisposition was suspected based on the high frequency of CVM in certain blood lines, however, it has not really been proven. Probably there is a genetic predisposition by inheriting an increased sensitivity to the environmental factors (Nout and Reed, 2003), such as high energy and high carbohydrate diet, high growth rates, copper deficiency, workload and trauma (Janes et al., 2015; Nout and Reed, 2003).

Probably, there are multiple pathways leading to CVM in horses, but the result is always characterized by static stenosis and/or dynamic, repetitive trauma to the spinal cord. This trauma causes direct injury to neurons and glia cells, but also secondary injury like ischemia, excitotoxicity and apoptosis (Baptiste and Fehlings, 2006; Karadimas et al., 2015).

The static or dynamic compression of the cervical spinal cord in CVM horses can be caused by osteosclerosis of the dorsal lamina, degenerative joint disease, osteochondrosis (OC), bone cysts, synovial cysts and thickening of the ligamentum flavum and joint capsule of the cervical vertebrae (Janes et al., 2015; Powers et al., 1986; Trostle et al., 1993). How these pathologies develop has been investigated several times and currently the most likely theory is a combination of developmental and biomechanical mechanisms (Janes et al., 2015). Abnormal development of cervical vertebrae during growth and maturation is certainly an important factor in the pathogenesis of CVM. The abnormal development can be caused by OC as it causes spinal canal abnormalities or contributes to instability of the cervical vertebrae (Trostle et al., 1993). However, OC is not a universal factor in CVM, as degenerative joint disease can be present without signs of OC (Trostle et al., 1993) and not all horses with osteochondrotic lesions develop CVM. Though, several studies have demonstrated a positive correlation between both diseases (Janes et al., 2015; Stewart et al., 1991; Trostle et al., 1993). Trauma might also be a factor in CVM development. In many CVM horses, there is a history of recent trauma. Trauma can cause excessive stretching of the joint capsule and ligamentum flavum or may damage the articular process joints, resulting in instability (Powers et al., 1986). Regardless of the cause, instability of the cervical vertebrae will lead to increased sensitivity to excessive or abnormal biomechanical forces and degenerative joint disease. The horses will develop osteosclerosis of the dorsal lamina, hypertrophy of the ligamentum flavum and joint capsule and osteophytosis of the articular process joints (Powers et al., 1986; Trostle et al., 1993).

IMPORTANCE, TREATMENT AND PROGNOSIS

With or without conservative treatment, the prognosis for CVM is poor to guarded because of continuing trauma to the spinal cord. Factors influencing prognosis are the age of the horse, a higher grade of ataxia, duration of the clinical signs and owner expectations (Bedenice and Johnson, 2018). Generally, horses with CVM will be able to live, but performance will be impaired. Conservative treatment is based on diminishing spinal cord compression by local and systemic anti-inflammatory drugs, decreasing growth speed with a low protein and low energy diet and limiting movement. Hoffman and Clark (2013) described that some thoroughbreds (about 30%) were able to race at least once after CVSM diagnosis. However, if

bony malformations or soft tissue proliferations exist, neurological deficits remain present (Hoffman and Clark, 2013; Levine et al., 2010). As a genetic basis is suspected, the affected horses are not suitable for breeding. Consequently, a lot of them are euthanized when the diagnosis of CVM is confirmed (Levine et al., 2008; Levine et al., 2010; Nout and Reed, 2003; Szklarz et al., 2018).

Surgical treatment can improve prognosis if the repetitive trauma to the spinal cord can be stopped. In studies in man (Kumar et al., 1999) and in dogs (Langerhuus and Miles, 2017), 80-90% of the patients with spinal cord compression showed improvement after surgery. However, as neurons do not regenerate easily, a (large) part of the present neuronal damage might be irreversible. So, surgical decompression may not result in complete normalization of the horses but adequate blood supply and resolution of inflammation and edema might improve neurological signs (Grant et al., 1979). In contrast to humans and dogs, improvement in horses, without complete normalization, is often not enough, knowing that the expectations for performance are very high. Moore et al. (1993) and Walmsley (2005) described that 45-60% of the patients who underwent surgery, did return to use. However, the prognosis is affected by case selection, duration of disease, the number of compression sites, severity of clinical signs and post-operative complications (Nout and Reed, 2003). So, the ideal candidate for surgery is a horse with recent onset of mild to moderate ataxia, responsive to corticosteroid therapy (Nixon and Stashak, 1983).

Two types of surgery have been described: the dorsal laminectomy and the cervical ventral body fusion. The dorsal laminectomy is performed in cases with static compression by a dorsal midline incision followed by removal of soft tissue, the caudal part of the dorsal lamina of the cranial vertebra and the cranial part of the dorsal lamina in the caudal vertebra (Nixon and Stashak, 1983). The surgery results in immediate decompression of the spinal cord, but is technically demanding and severe complications are common (Nout and Reed, 2003). In literature it has been described that 40-75% of the laminectomies result in neurological improvement of 1-3 grades. However, these studies also mention that 25-40% of the horses are euthanized or have more severe neurological signs after surgery, often caused by additional fracture of the articular process joints (Moore et al., 1993; Nixon and Stashak, 1983).

The ventral body fusion is used to stabilize dynamic compressive lesions but can also relieve static compression. By stabilization of the vertebra, mechanical stress and proliferative growth decreases, resulting in remodeling of the articular process joints and decompression of the spinal cord (Moore et al., 1993). The surgery is less demanding and fixates the affected vertebrae in extended position to prevent repetitive compression and trauma to the spinal cord. Most commonly, the intervertebral disc is removed and a stainless steel Basket or Kerf Cut Cylinder, packed with an autogenous bone graft, is implanted from the ventral aspect of the vertebra to induce arthrodesis of the cervical vertebrae (Huggons, 2007; Moore et al., 1993; Nout and Reed, 2003; Smyth, 1993). The use of a ventral locking compression plate also delivers good results (Kuhnle et al., 2018; Reardon et al., 2009) and might be biomechanically superior compared to the Kerf Cut Cylinder implantation (Reardon et al., 2010). General success rate of ventral body fusion, defined as improvement of at least 1 neurological grade, is about 70-75%. About 5-12% of the horses do not show improvement and another 12-20% suffer from severe complications and need to be euthanized (Kuhnle et al., 2018; Moore et al., 1993; Walmsley, 2005). However, a recent study on a limited number (n=14) of horses reported euthanasia in 57% of all surgical cases (Szklarz et al., 2018). The most important complications are cervical fractures, implant infections and migration, but also screw migration, seroma development, colitis, right laryngeal hemiplegia and plate breakage have been reported (Kuhnle et al., 2018; Moore et al., 1993; Walmsley, 2005).

DIAGNOSIS

There is currently no gold standard for diagnosis of CVM. So, decision making is mostly based on a combination of different examinations and exclusion of possible differential diagnoses. An overview of the commonly used diagnostic tests, such as the clinical examination and diagnostic imaging techniques (cervical radiography, myelography), and more advanced or innovative approaches, such as vertebral canal endoscopy and electrophysiological tests, are given in the following paragraphs.

CLINICAL NEUROLOGICAL EXAMINATION

Taking the signalement and detailed history of the patient and performing a complete neurological examination is the first step in the diagnostic process. The (normal) behaviour and posture of the horse need to be assessed, as well as the position of the head and the cranial nerves. Subsequently, the coordination of the horses is evaluated during walk and trot on a straight line and on circles, during canter and transitions. Turning small circles, backing, walking with the head elevated or blindfolded or walking over an obstacle or on a slope might emphasize subtle clinical signs. Special attention should be paid to symmetry of the movements, weakness, spasticity or ataxia and if abnormalities are present in 1 or more limbs. Additionally, tail pull and crossing over of the limbs can be performed (Alcott, 2017; Olsen et al., 2014; Saville et al., 2017).

The goal of the neurological examination is to determine if there is neurological disease and to localize the neurological lesion (cerebrum, cerebellum, brain stem, spinal cord or peripheral nerves) and hence, to navigate subsequent medical imaging in the right direction. However, the neurological examination remains subjective and even between skilled observers there is only poor to fair agreement, certainly in the more subtle cases (Olsen et al., 2014; Saville et al., 2017).

DIAGNOSTIC IMAGING

Cervical radiographs

Lateral radiographs of the cervical vertebrae can be made easily in the standing horse. These radiographs are evaluated for vertebral malformation or vertebral canal stenosis as indication for CVM, but the value of this examination remains controversial. Vertebral malformation is evaluated subjectively by the presence of physitis of the caudal vertebral body, caudal extension of the dorsal arch of the vertebral body, intervertebral malalignment and arthropathy and/or osteochondrosis of the articular process joints (Mayhew et al., 1978; Papageorges et al., 1987). Although these bony malformations are seen more in CVM horses, on their own, they offer poor positive and negative predictive values and do not distinguish between normal and CVM horses (Moore et al., 1994; Papageorges et al., 1987). Certainly the articular process joints show often degenerative joint disease in neurologically normal horses

too (Down and Henson, 2009; Moore et al., 1994). Therefore, Mayhew et al. (1993) developed a total CVM score to diagnose and predict CVM more accurate in thoroughbred foals. This score combines the bony malformations mentioned above with parameters for vertebral canal stenosis. The most contributing factors to distinguish normal and affected horses were angulation of the vertebrae and stenosis of the vertebral canal.

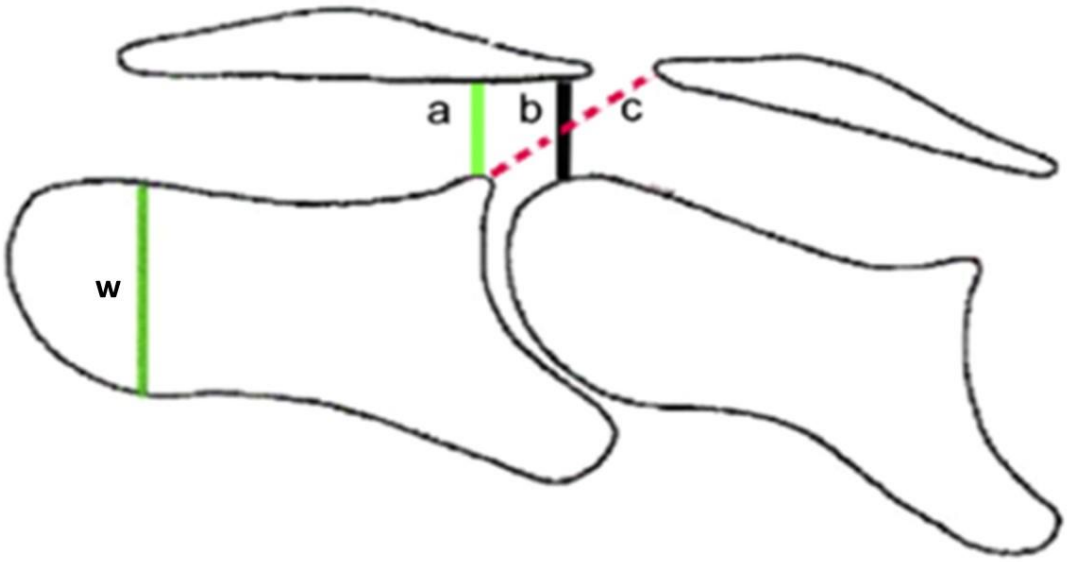


Figure 3. Representation of measuring sites for intra- and intervertebral ratio calculations. The intravertebral ratio is calculated by dividing the sagittal diameter of the spinal canal (distance a) and the maximal height of the cranial part of the vertebral body (distance w). The intervertebral ratio is calculated by dividing the distance between the most caudal point of the dorsal lamina and the cranial part of the sequential vertebral body (distance b) or the most caudal point of the vertebral body and the cranial part of the sequential dorsal lamina (distance c) by the vertebral body width (w) (from Hahn et al. (2008)).

The most reliable method to assess canal stenosis is calculation of sagittal ratios by dividing the sagittal diameter of the vertebral canal by the maximal height of the cranial part of the vertebral body (Figure 3). By using this ratio, the absolute diameter of the vertebral canal is corrected for radiographic magnification and the size of the horses (Moore et al., 1994). The ideal cut-off value varies depending on the study from 0.485 to 0.52 for C4, C5 and C6 and from 0.485 to 0.56 for C7 (Hahn et al., 2008; Moore et al., 1994). Reported sensitivity and

specificity values vary, depending on the study and the used cut-off value (table 3). Furthermore, measurement errors, within and between observers, may limit diagnostic accuracy (Hughes et al., 2014). So, to date, plain radiographs might indicate CVM but additional examinations are required for definite diagnosis.

Myelography

For myelography, contrast medium is injected in the subarachnoidal space surrounding the spinal cord. Nowadays, mostly iohexol at a dosage of 30-35mg/kg is used (Estell et al., 2018; Mullen et al., 2015; Szklarz et al., 2018; Walmsley, 2005) as it is a safer contrast medium than the formerly used metrizamide (Widmer et al., 1998). Before injection, a similar volume of CSF needs to be withdrawn. This CSF punction and contrast injection can be performed blindly or ultrasound guided. By visualizing the needle by ultrasound, the amount of complications of contrast injection, such as epi- or subdural contrast injection or blood contamination by puncture of the lateral venous sinus, can be minimized (Audigie et al., 2004). After contrast injection, the head is elevated during 5 minutes and then lateral radiographs of the neck in neutral, flexed and extended position are taken to evaluate the spinal canal and sites of compression (Figure 4). The technique is considered to be save with a low percentage of mostly mild and limited adverse effects. Reported adverse effects are fever, hyperesthesia, seizures, muscle spasms and exacerbation of neurologic deficits (Mullen et al., 2015; Papageorges et al., 1987). Ideally, the procedure is performed under general anaesthesia. A standing myelography is possible, but has limited utility as it might provoke adverse effects or discomfort to the horses and no proper extension and flexion of the neck is possible in the standing horse (Rose et al., 2007). However, this flexion and extension are really important as most CVM horses show dynamic stenosis (Papageorges et al., 1987; Szklarz et al., 2018).

Myelography is currently still considered the best ante mortem test available to diagnose CVM or spinal cord compression in general (Hahn et al., 2008; Levine et al., 2007; Papageorges et al., 1987; Szklarz et al., 2018; van Biervliet et al., 2004). Historically used criteria for diagnosis of CVM are at least 50% reduction of the dorsal (and ventral) contrast column, a reduction of the dural diameter of at least 20% or a dorsal contrast column of less than 2mm (Papageorges et al., 1987; van Biervliet et al., 2004). The 2mm rule is nowadays not recommended because of influence of differences in anatomy and magnification. The other criteria have high

sensitivities and specificities at the level of C6-7, but are known to have low sensitivity and moderate specificity at other cervical levels (table 3). Flexion of the neck will increase sensitivity but will also increase the number of false positives (van Biervliet et al., 2004). An explanation for the low sensitivity might be a high percentage of CVM horses with dorsolateral compression due to enlarged articular process joints which will not be visible on the lateral view (Schmidburg et al., 2012; van Biervliet et al., 2004). Furthermore, it is possible that the spinal cord itself is swollen or atrophied because of the compression and so influencing the measurements (van Biervliet et al., 2004). So, although myelography might currently be the best available test, it cannot be used as gold standard.

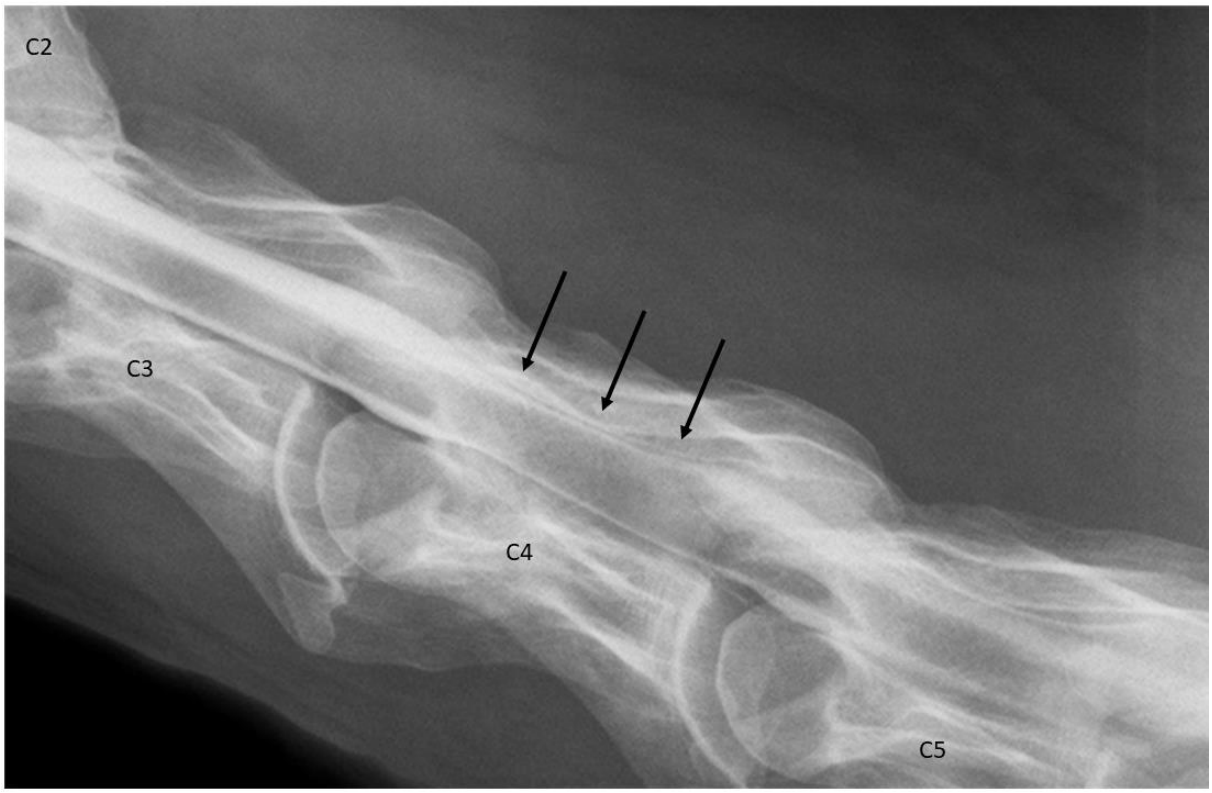


Figure 4: Myelogram at the level of the third to fifth cervical vertebra with >50% dorsal and ventral reduction of contrast column (indicated by arrows) at the level of C4.

Table 3a. Overview of reported sensitivity and specificity values for the most commonly used cut-off values for cervical radiography for CVM diagnosis.

Cervical radiography			
Used cut-off	Sensitivity	Specificity	Specificity
Intravertebral ratio 0.52-0.56	89%	89%	(Moore et al., 1994)
Intravertebral ratio 0.52-0.56	47%	78%	(Levine et al., 2007)
Intravertebral ratio 0.50-0.52	42%	83%	(Levine et al., 2010)

Table 3b. Overview of reported sensitivity and specificity values for the most commonly used cut-off values for myelography for CVM diagnosis.

Myelography			
Used cut-off	Sensitivity	Specificity	Specificity
20% reduction dural diameter	33-100%	27-100%	(van Biervliet et al., 2004)
50% reduction dorsal contrast column	53%	89%	(van Biervliet et al., 2004)

CT and MRI

These advanced imaging techniques are frequently used in small animal veterinary and human medicine to diagnose spinal cord disease. A couple of studies describe the normal anatomy of the cervical spine in horses after euthanasia (Sleutjens et al., 2014; Veraa et al., 2016). For diagnosis of CVM in living horses, there is limited information available, probably because of the low availability of big-bore CT or MRI gantries. Because of the size of the horses, imaging of the neck is often limited to the cranial parts (Szklarz et al., 2018). To our knowledge, there is only 1 study reporting findings in living foals with spinal cord compression. The foals were maximally 33 weeks old, but because of the small CT diameter, it was not possible to scan the caudal cervical vertebrae in all foals (Yamada et al., 2016). However, the technique was sensitive to detect lesions and provides interesting perspectives for the future if bigger scan gantries become more widely available (Moore et al., 1992; Yamada et al., 2016).

MRI has some interesting advantages compared to the formerly discussed techniques. MRI is superior to visualize soft tissue, 3D images are made and it even does not require inthrathecal contrast administration. However, concerning cervical MRI in horses, only post mortem examinations are published, again because of the size limitations. In these studies, MRI has moderate sensitivity and good specificity to subjectively (Mitchell et al., 2012) or quantitatively (Janes et al., 2014) detect spinal cord compression in horses. False negatives might occur because of dynamic compression which could not be identified in neutral position. False positives might result from lesion causing functional deficits without histopathological changes (Mitchell et al., 2012). Concerning detection of the site of compression, result are contradictory. Mitchell et al. (2012) stated that the compression site could not be identified while Janes et al. (2014) stated that the vertebral canal was smaller at the compressive sites and thus enabled identification of compression. Although the technique is not practically useable in horses yet, it might become interesting in the future if further research is performed.

HISTOPATHOLOGY

Histopathology is only possible post mortem, so it cannot help in decision making (surgery, euthanasia, conservative treatment,..) in living horses, but it is considered the “gold standard” for CVM diagnosis (Nout and Reed, 2003; Szklarz et al., 2018).

At the level of compression, focal degenerative lesions such as neuronal fiber swelling and degeneration, occasional spheroids, increased macrophage activity, astrocytic gliosis and myelin degeneration or loss can be found in grey and more prominent in the white matter on hematoxylin eosin stain (Furr and Reed, 2008). Secondary Wallerian degeneration of the white matter will be present. Wallerian degeneration is a trophic degeneration that originates at the site of the lesion and travels in distal direction. Therefore it is seen in the ascending pathways above and the descending pathways below the lesion (De Lahunta and Glass, 2009).

This typical distribution of degenerative lesions enables differentiation between compressive causes and other etiologies of general proprioceptive ataxia such as equine degenerative myelencephalopathy and equine protozoal myeloencephalitis. In equine degenerative myelencephalopathy, there is no focal lesion but degeneration of ascending and descending

white matter tract throughout the spinal cord, particularly in the superficial lateral and ventromedial pathways of the thoracic segment. Selective dorsal gray column degeneration can be found too (De Lahunta and Glass, 2009, Mayhew 2008). With equine protozoal myelencephalitis, inflammatory, asymmetric and multifocal lesions are affecting both white and grey matter.

To accentuate lesions, additional staining can be used. The luxol fast blue stain for instance will demonstrate demyelination as it colors myelin, but also specific immunohistochemical stains can be used (De Lahunta and Glass, 2009).

OTHER (CSF, ENDOSCOPY)

In horses with CVM, cerebrospinal fluid (CSF) is normal in the majority of cases. It might show higher creatinine kinase activity, but sensitivity and specificity is too low (less than 60%) to be of practical use (Jackson et al., 1996). High resolution electrophoresis and the presence of post beta peaks can be used for CVM diagnosis with moderate (70-80%) sensitivity and specificity (Furr et al., 1997) and the fluid might have a slightly increased protein concentration and mild xantochromia. CSF analysis is particularly interesting to rule out inflammatory causes and might give an indication, but mostly, it will not be conclusive for the diagnosis of CVM.

Cervical vertebral canal endoscopy under general anesthesia has been experimentally used in horses with CVM. Myeloscopy could successfully identify the site of spinal cord compression but as it is a subjective evaluation, the technique might not be reliable in mild to moderate narrowing of the spinal canal. Furthermore, the procedure is invasive and dynamic examination is not possible as this is too dangerous to damage the spinal cord during manipulation (Prange et al., 2012). During the procedure, life threatening complications such as trauma of the spinal cord and hemorrhage are possible (Prange et al., 2011) making it not the ideal diagnostic test.

NEUROFUNCTIONAL TESTING (ELECTROMYOGRAPHY (EMG), MMEP)

Medical imaging is interesting to demonstrate structural changes, but, as mentioned above, it does not always discriminate between functional and subclinical lesions. Therefore, it is interesting to correlate results of medical imaging with neurophysiological examinations of the spinal cord (Chan et al., 1998; Nardone et al., 2014).

Needle electromyography is used in horses to make a differentiation between neuropathy and myopathy. With paraspinal muscle examination, it should also be possible to grossly localize a lesion (Wijnberg, 2005; Wijnberg et al., 2004). However, EMG only provides information about the lower motor neurons and no information about the nerve conduction through the spinal cord. Transcranial magnetic stimulation (TMS) and transcranial electrical stimulation (TES) are 2 techniques who do give information about the spinal cord motor function. TES has only been described in normal horses so far (Journée, 2014; Journee et al., 2015, 2018), but might be useable in future. TMS has been described in normal horses and horses with ataxia or in recumbency (Nollet et al., 2002; Nollet et al., 2004; Nollet et al., 2003a; Nollet et al., 2003c; Nollet et al., 2005). Also in dogs and in humans, TMS is used to evaluate spinal cord motor function.

USE OF TMS

USE OF TMS IN HUMAN MEDICINE TO EVALUATE SPINAL CORD FUNCTION

In human medicine the diagnostic utility of TMS for myelopathy is confirmed. TMS can diagnose and determine the level of spinal cord compression. MMEPs are recorded using surface electrodes on the target muscles. Frequently used target muscles are the abductor digiti minimi and the extensor carpi radialis in the upper limbs and the anterior tibial muscle or abductor halluces muscle in the lower limbs (Funaba et al., 2015; Groppa et al., 2012; Nakamae et al., 2010; Nakanishi et al., 2014). However, other target muscles are possible too. Intramuscular needle recordings of paraspinal muscles may help to determine the exact level of a spinal lesion (Ertekin et al., 1998) but also facial, respiratory, laryngeal, pharyngeal and pelvic muscles can be evaluated using TMS (Groppa et al., 2012). The contraindications are similar to those of MRI. Main risks are ferromagnetic implants or pacemakers. TMS also might induce epilepsy but the risk is very low (Wassermann, 1998).

TMS induces a desynchronized excitation of the muscle fibers resulting in a polyphasic MMEP. The degree of desynchronization is usually larger for lower limb muscles but varies from stimulation to stimulation because of intrinsic changes in excitability of the motor cortex and spinal motor neurons. Therefore, it is advised to record at least 5-6 MMEPs and to choose the

one with the largest amplitude and shortest latency as this reflects the optimal conduction (Groppa et al., 2012).

To evaluate the function of the spinal cord in human medicine, the central motor conduction time (CMCT) is used (Chen et al., 2008). CMCT is an estimation of the conduction time between the motor cortex and the spinal motor neurons. By using this parameter, instead of cortex to muscle ratio, a differentiation between central and peripheral nerve disorders can be made. CMCT is calculated by subtracting the peripheral conduction time (spinal motor neuron to muscle latency time) from the cortex to muscle latency. The peripheral conduction time is estimated using F-waves or motor root stimulation (Chen et al., 2008). F-waves are elicited by strong electrical stimulation of peripheral nerves. This pulse travels in distal direction and evokes a direct muscle contraction (M-wave) but also travels in proximal direction where the spinal motor neurons are activated and send a pulse back to distal to evoke a second muscle contraction. This late and smaller muscle contraction is called the F-wave. The peripheral conduction time is then calculated as (F+M-1)/2 (Chen et al., 2008). The second method, the motor root stimulation can be performed by magnetic or electrical stimulation over the vertebral column to excite the motor roots at their *exit foramina*. The pulse travels distally and evokes a muscle contraction. The time between stimulation and contraction estimates the peripheral conduction time. The technique is easier to perform, but underestimates the peripheral conduction time as the proximal part of the nerve root is not included (Mills and Murray, 1986).

To measure the optimal shortest CMCT, TMS needs to be performed with an active target muscle (facilitation principle) as the motor neurons are “close to firing” and very susceptible for stimuli then (Chen et al., 2008). Age and height have an influence on latency. In adults, there is no clear or only small correlation with age (Imajo et al., 2017; Mano et al., 1992), but in neonates and children responses are often difficult to obtain (Claus, 1990). An effect of height is evident as latency time will be longer if the conduction pathway is longer. As the distance to the cervical vertebrae is a lot shorter than to the lumbar region, the strongest influence is found for the latter and there is only a weak correlation between height and the upper limb muscle latencies (Claus, 1990; Imajo et al., 2017).

In general, upper limb MMEPs are easier to evoke than lower limb MMEPs. The cortical zone which need to be stimulated to evoke a contraction in the lower limb muscles is situated a lot deeper in the brain and because of the longer conduction distance, there is more temporal dispersion of the descending volleys (Groppa et al., 2012).

CMCT is correlated with the severity of compression on MRI (Lo et al., 2004) and with the degree of functional involvement of the spinal cord. Moreover, TMS has a sensitivity and specificity of 100% and 84.5% to detect cord compression (Lo et al., 2004) and might even detect subclinical lesions (Travlos et al., 1992). Furthermore, it can determine which sites with anatomical cord compression result in really functional lesions, better than MRI (Chan et al., 1998; Deftereos et al., 2009; Di Lazzaro et al., 1992). Serial MMEP recording allows identifying progressive disease and pre- and post-operative testing provides information about the result of surgery (Nakanishi et al., 2014; Nardone et al., 2016). However, pre-surgical TMS cannot determine the cause of the lesion (neoplastic compression, myelitis, MS, motor neuron disease,…) nor predict clinical outcome after treatment (Brunholzl and Claus, 1994; Jaskolski et al., 1990; Nardone et al., 2016).

USE OF TMS IN DOGS

In dogs, the use of TMS has been described since 1990 (Kraus et al., 1990). The technique was developed in normal sedated (Van Ham et al., 1994) and anesthetized dogs (Van Ham et al., 1996a; Van Ham et al., 1996b) and has been used to assess spinal cord integrity in dogs with intervertebral disc and spinal cord disease (Poma et al., 2002; Sylvestre et al., 1993). Mostly, TMS is performed on heavily sedated dogs. Sedation is achieved using a combination of acepromazine and an opioid and the dogs are placed in lateral or sternal recumbency (Amendt et al., 2017; da Costa et al., 2006; De Decker et al., 2011; Poma et al., 2002; Van Ham et al., 1994). Magnetic stimulation is performed centrally on the vertex of the dogs with 4 repetitions per recording site (Amendt et al., 2017). MMEP recording sites are the extensor carpi radialis (ECR) and tibilialis cranialis (TC) muscle. For recording of MMEPs, intramuscular needle electrodes are used and the parameters to evaluate are latency and amplitude (Amendt et al., 2017; da Costa et al., 2006; De Decker et al., 2011; Martin-Vaquero and da Costa, 2014; Van Ham et al., 1994).

In dogs with spinal cord lesions, a significant attenuation of latency and amplitude values was found and in several severely diseased dogs, MMEPs could even not be registered. Poma et al. (2002), da Costa et al. (2006) and Martin-Vaquero and da Costa (2014) found a correlation between MMEP latencies and the severity of clinical signs: the more severe the neurologic deficits, the more prolonged the latencies but, Amendt et al. (2017) could not confirm these findings in their study. They also found prolonged MMEP latencies in dogs with and absent MMEP in dogs without preserved motor function, but there were no significant differences between dogs with different severities of neurological signs. Also, dogs with spinal pain, without neurologic deficits, showed significantly increased latencies (Amendt et al., 2017).

Several studies compared findings of TMS with those of MRI in dogs with and without spinal cord disease (da Costa et al., 2006; De Decker et al., 2011; Martin-Vaquero and da Costa, 2014). MRI is an excellent technique to define anatomic lesions, but it does not provide information about spinal cord function and there is a risk of over-interpretation. Therefore, TMS is suggested to be a valuable additional test to help clinicians to identify relevant spinal cord compression (De Decker et al., 2011). Da Costa et al. (2006) found that latencies in ECR muscle were not different between normal and diseased dogs but there was a correlation between TC muscle latencies and MRI findings in dogs with cervical spondylomyelopathy. However, not all MRI findings had clinical importance. Some dogs who showed spinal cord compression on MRI were clinically normal and had normal latency values. In other dogs with spinal compression, latency time was already prolonged, despite the absence of clinical signs. So, TC MMEP latency appeared to be a sensitive test and was useable to assess spinal cord function in doubtful compression sites on MRI or as a screenings test. Additionally, there were no dogs with neurological deficits and normal TC latencies, indicating a high specificity of TC MMEPs. De Decker et al. (2011) and Martin-Vaquero and da Costa (2014) confirmed the correlation between TC muscle latency and MRI compression but also found a significant correlation between ECR and TC latency and the severity of spinal cord compression. Sensitivity and specificity values to discriminate between clinically normal and affected dogs, are clearly higher for the TC MMEP latencies than for EC MMEP latencies (De Decker et al., 2011).

TMS can also be used to evaluate the effect of surgery, but there is a delay in spinal cord function recovery. Three to six months after surgery might still be too early for TMS evaluation as re-myelination can occur at later stages. However, it is possible that MMEPs never return to normal if definite axon damage is present (Amendt et al., 2017).

USE OF TMS IN HORSES

TMS in horses was first described by Mayhew and Washbourne (1996). Much later, the technique was standardized in horses (Nollet et al., 2003a). Unlike in dogs, a combination of detomidine and an opioid was used to sedate the horses. This combination results in a sufficient sedation state and did not influence onset latency and peak-to-peak amplitude in horses (Nollet et al., 2003b). The horses were stimulated with a circular 70mm coil generating a maximal capacity of 4 Tesla. Coil current did not change measured results but coil position was an important factor. Best MMEPs were recorded with the coil positioned axial on the cranium, on or just underneath the line between both ears bases and 100% stimulus output. Like in dogs, MMEPs were recorded using intramuscular needle electrodes in the TC muscle in the pelvic and the EC muscle in the thoracic limbs. The ground electrode was placed at the skin in the groin and elbow region, respectively (Nollet et al., 2003a). Using this standardized technique, normal values for onset latency and peak-to-peak amplitude were 19.32 ± 2.50 ms and 30.54 ± 5.28 ms and 9.52 ± 3.73 mV and 6.62 ± 3.62 mV in thoracic and pelvic limbs, respectively. There was no significant difference between left and right side measurements nor between different sexes. Horse age had a small significant effect on peak-to-peak amplitude and height and weight had an important influence on latency. In general, latency had a low variability in contrast to amplitude measurements which were very variable, even within the same muscle (Nollet et al., 2004).

Clinically, TMS has proven its utility in horses with bilateral hind limb ataxia (Nollet et al., 2003c), cervical spinal cord disease (Nollet et al., 2002) and to differentiate between neurological and other causes of recumbency (Nollet et al., 2005). Latency values are prolonged and amplitude values decreased compared to the normal reference values. If both thoracic and pelvic limb MMEPs are abnormal, a cervical lesion is suspected whereas a more caudal localization is suspected if only pelvic limb MMEPs are abnormal (Nollet et al., 2003c).

In some cases, diagnosis could be confirmed with necropsy but in several cases, a definite diagnosis was never made as there is no gold standard in living horses. Also, data about the agreement between TMS and cervical radiographs or other imaging techniques are not available. However, as cervical radiographs often do not show any abnormalities, despite clinical evidence of cervical spinal cord disease, while TMS does, the latter seems more sensitive. However, real sensitivity and specificity values could not be determined yet.

CONCLUSION

Despite the importance of spinal cord disease, the diagnosis remains difficult in living horses. There is a need for easy and quick screening tests but also tests to confirm the presumptive diagnosis are lacking. Advanced imaging techniques may improve the diagnostic accuracy in the future, but the risk of over interpretation is real. Therefore, TMS-MMEP is an interesting additional test, offering a lot of opportunities. However, the possibilities are still limited and the validation is incomplete.

REFERENCES

Alcott, C.J., 2017. Evaluation of ataxia in the horse. Equine Veterinary Education 29, 629-636.

Amendt, H.L., Siedenburg, J.S., Steffensen, N., Kordass, U., Rohn, K., Tipold, A., Stein, V.M., 2017. Correlation between severity of clinical signs and transcranial magnetic motor evoked potentials in dogs with intervertebral disc herniation. Veterinary Journal 221, 48-53.

Audigie, F., Tapprest, J., Didierlaurent, D., Denoix, J.M., 2004. Ultrasound-guided atlanto-occipital puncture for myelography in the horse. Veterinary Radiology and Ultrasound 45, 340-344.

Baptiste, D.C., Fehlings, M.G., 2006. Pathophysiology of cervical myelopathy. The Spine Journal 6, 190S-197S.

Bedenice, D., Johnson, A.L., 2018. Neurologic Conditions Affecting the Equine Athlete. The Veterinary clinics of North America. Equine practice 34, 277-297.

Brunholzl, C., Claus, D., 1994. Central motor conduction time to upper and lower limbs in cervical cord lesions. Archives of neurology 51, 245-249.

Chan, K.M., Nasathurai, S., Chavin, J.M., Brown, W.F., 1998. The usefulness of central motor conduction studies in the localization of cord involvement in cervical spondylytic myelopathy. Muscle & nerve 21, 1220-1223.

Chen, R., Cros, D., Curra, A., Di Lazzaro, V., Lefaucheur, J.P., Magistris, M.R., Mills, K., Rosler, K.M., Triggs, W.J., Ugawa, Y., Ziemann, U., 2008. The clinical diagnostic utility of transcranial magnetic stimulation: report of an IFCN committee. Clinical Neurophysioly 119, 504-532.

Claus, D., 1990. Central motor conduction: method and normal results. Muscle & nerve 13, 1125-1132.

da Costa, R.C., Poma, R., Parent, J.M., Partlow, G., Monteith, G., 2006. Correlation of motor evoked potentials with magnetic resonance imaging and neurologic findings in Doberman Pinschers with and without signs of cervical spondylomyelopathy. American Journal of Veterinary Research 67, 1613-1620.

De Decker, S., Van Soens, I., Duchateau, L., Gielen, I.M.V.L., van Bree, H.J.J., Binst, D.H.A.R., Waelbers, T., Van Ham, L.M.L.M., 2011. Transcranial magnetic stimulation in Doberman Pinschers with clinically relevant and clinically irrelevant spinal cord compression on magnetic resonance imaging. Journal of the American Veterinary Medical Association 238, 81-88.

Deftereos, S.N., Kechagias, E.A., Panagopoulos, G., Seretis, A., Orphanidis, G., Antoniou, E., Georgakoulias, N., Karageorgou, C.E., 2009. Localisation of cervical spinal cord compression by TMS and MRI. Functional neurology 24, 99-105.

De Lahunta, A., Glass, E., Veterinary neuroanatomy and clinial neurology. 3rd Edition. Missouri: Saunders; 2009.

Di Lazzaro, V., Restuccia, D., Colosimo, C., Tonali, P., 1992. The contribution of magnetic stimulation of the motor cortex to the diagnosis of cervical spondylotic myelopathy. Correlation of central motor conduction to distal and proximal upper limb muscles with clinical and MRI findings. Electroencephalography and Clinical Neurophysiology 85, 311-320.

Down, S.S., Henson, F.M., 2009. Radiographic retrospective study of the caudal cervical articular process joints in the horse. Equine Veterinary Journal 41, 518-524.

Ertekin, C., Uludag, B., On, A., Yetimalar, Y., Ertas, M., Colakoglu, Z., Arac, N., 1998. Motor-evoked potentials from various levels of paravertebral muscles in normal subjects and in patients with focal lesions of the spinal cord. Spine 23, 1016-1022.

Estell, K., Spriet, M., Phillips, K.L., Aleman, M., Finno, C.J., 2018. Current dorsal myelographic column and dural diameter reduction rules do not apply at the cervicothoracic junction in horses. Veterinary Radiology and Ultrasound 59, 662-666.

Funaba, M., Kanchiku, T., Imajo, Y., Suzuki, H., Yoshida, Y., Nishida, N., Taguchi, T., 2015. Transcranial magnetic stimulation in the diagnosis of cervical compressive myelopathy: comparison with spinal cord evoked potentials. Spine 40, 161-167.

Furr, M., Chickering, W.R., Robertson, J., 1997. High resolution protein electrophoresis of equine cerebrospinal fluid. American Journal of Veterinary Research 58, 939-941.

Furr, M., Reed, S. Equine neurology. Iowa: Blackwell publishing; 2008.

Groppa, S., Oliviero, A., Eisen, A., Quartarone, A., Cohen, L.G., Mall, V., Kaelin-Lang, A., Mima, T., Rossi, S., Thickbroom, G.W., Rossini, P.M., Ziemann, U., Valls-Sole, J., Siebner, H.R., 2012. A practical guide to diagnostic transcranial magnetic stimulation: report of an IFCN committee. Clinical Neurophysioly 123, 858-882.

Hahn, C.N., Handel, I., Green, S.L., Bronsvoort, M.B., Mayhew, I.G., 2008. Assessment of the utility of using intra- and intervertebral minimum sagittal diameter ratios in the diagnosis of cervical vertebral malformation in horses. Veterinary Radiology and Ultrasound 49, 1-6.

Hamir, A.N., Moser, G., Rupprecht, C.E., 1992. A five year (1985-1989) retrospective study of equine neurological diseases with special reference to rabies. Journal of comparative pathology 106, 411-421.

Hoffman, C.J., Clark, C.K., 2013. Prognosis for racing with conservative management of cervical vertebral malformation in thoroughbreds: 103 cases (2002-2010). Journal of Veterinary Internal Medicine 27, 317-323.

Huggons, N., 2007. Tri-level surgical treatment of cervical spinal cord compression in a Thoroughbred yearling. The Canadian Veterinary Journal = La revue veterinaire canadienne 48, 635-638.

Hughes, K.J., Laidlaw, E.H., Reed, S.M., Keen, J., Abbott, J.B., Trevail, T., Hammond, G., Parkin, T.D., Love, S., 2014. Repeatability and intra- and inter-observer agreement of cervical vertebral sagittal diameter ratios in horses with neurological disease. Journal of Veterinary Internal Medicine 28, 1860-1870.

Imajo, Y., Kanchiku, T., Suzuki, H., Yoshida, Y., Funaba, M., Nishida, N., Fujimoto, K., Taguchi, T., 2017. Effects of differences in age and body height on normal values of central motor conduction time determined by F-waves. The journal of spinal cord medicine 40, 181-187.

Jackson, C., de Lahunta, A., Divers, T., Ainsworth, D., 1996. The diagnostic utility of cerebrospinal fluid creatine kinase activity in the horse. Journal of Veterinary Internal Medicine 10, 246-251.

Janes, J.G., Garrett, K.S., McQuerry, K.J., Pease, A.P., Williams, N.M., Reed, S.M., MacLeod, J.N., 2014. Comparison of magnetic resonance imaging with standing cervical

radiographs for evaluation of vertebral canal stenosis in equine cervical stenotic myelopathy. Equine Veterinary Journal 46, 681-686.

Janes, J.G., Garrett, K.S., McQuerry, K.J., Waddell, S., Voor, M.J., Reed, S.M., Williams, N.M., MacLeod, J.N., 2015. Cervical Vertebral Lesions in Equine Stenotic Myelopathy. Veterinary pathology 52, 919-927.

Jaskolski, D.J., Laing, R.J., Jarratt, J.A., Jukubowski, J., 1990. Pre- and postoperative motor conduction times, measured using magnetic stimulation, in patients with cervical spondylosis. British journal of neurosurgery 4, 187-192.

Journée, S.L., Delesalle, C.J.G., de Bruijn, C.M., Bergmann, W. and Journée, H.L., 2014. Transcranial electrical stimulation (TES) as a possible novel alternative to transcranial magnetic stimulation (TMS) to assess the motor function of the spinal cord for clinical diagnosis in horses. Equine Veterinary Journal 46, Suppl. 47, 10.

Journee, S.L., Journee, H.L., de Bruijn, C.M., Delesalle, C.J.G., 2015. Design and Optimization of a Novel Method for Assessment of the Motor Function of the Spinal Cord by Multipulse Transcranial Electrical Stimulation. in Horses. Journal of Equine Veterinary Science 35, 793-800.

Journee, S.L., Journee, H.L., de Bruijn, C.M., Delesalle, C.J.G., 2018. Multipulse transcranial electrical stimulation (TES): normative data for motor evoked potentials in healthy horses. BMC veterinary research 14.

Karadimas, S.K., Gatzounis, G., Fehlings, M.G., 2015. Pathobiology of cervical spondylotic myelopathy. European Spine Journal 24, S132-S138.

Kraus, K.H., O'Brien, D., Pope, E.R., Kraus, B.H., 1990. Evoked potentials induced by transcranial stimulation in dogs. American Journal of Veteriary Research 51, 1732-1735.

Kuhnle, C., Furst, A.E., Ranninger, E., Sanchez-Andrade, J.S., Kummerle, J.M., 2018. Outcome of Ventral Fusion of Two or Three Cervical Vertebrae with a Locking Compression Plate for the Treatment of Cervical Stenotic Myelopathy in Eight Horses. Veterinary and Comparative Orthopaedics and Traumatology 31, 356-363.

Kumar, V.G., Rea, G.L., Mervis, L.J., McGregor, J.M., 1999. Cervical spondylotic myelopathy: functional and radiographic long-term outcome after laminectomy and posterior fusion. Neurosurgery 44, 771-777; discussion 777-778.

Langerhuus, L., Miles, J., 2017. Proportion recovery and times to ambulation for non-ambulatory dogs with thoracolumbar disc extrusions treated with hemilaminectomy or conservative treatment: A systematic review and meta-analysis of case-series studies. Veterinary Journal 220, 7-16.

Levine, J.M., Adam, E., MacKay, R.J., Walker, M.A., Frederick, J.D., Cohen, N.D., 2007. Confirmed and presumptive cervical vertebral compressive myelopathy in older horses: A retrospective study (1992-2004). Journal of Veterinary Internal Medicine 21, 812-819.

Levine, J.M., Ngheim, P.P., Levine, G.J., Cohen, N.D., 2008. Associations of sex, breed, and age with cervical vertebral compressive myelopathy in horses: 811 cases (1974-2007). Journal of American Veterinary Medicine A 233, 1453-1458.

Levine, J.M., Scrivani, P.V., Divers, T.J., Furr, M., Mayhew, I.J., Reed, S., Levine, G.J., Foreman, J.H., Boudreau, C., Credille, B.C., Tennent-Brown, B., Cohen, N.D., 2010. Multicenter case-control study of signalment, diagnostic features, and outcome associated with cervical vertebral malformation-malarticulation in horses. Journal of the American Veterinary Medical Association 237, 812-822.

Lo, Y.L., Chan, L.L., Lim, W., Tan, S.B., Tan, C.T., Chen, J.L., Fook-Chong, S., Ratnagopal, P., 2004. Systematic correlation of transcranial magnetic stimulation and magnetic resonance imaging in cervical spondylotic myelopathy. Spine 29, 1137-1145.

Mano, Y., Nakamuro, T., Ikoma, K., Sugata, T., Morimoto, S., Takayanagi, T., Mayer, R.F., 1992. Central motor conductivity in aged people. Internal medicine 31, 1084-1087.

Martin-Vaquero, P., da Costa, R.C., 2014. Transcranial magnetic motor evoked potentials in Great Danes with and without clinical signs of cervical spondylomyelopathy: Association with neurological findings and magnetic resonance imaging. Veterinary Journal 201, 327-332.

Mayhew, I.G., de Lahunta, A., Whitlock, R.H., Krook, L., Tasker, J.B., 1978. Spinal cord disease in the horse. The Cornell veterinarian 68 Suppl 6, 1-207.

Mayhew, I.G., Donawick, W.J., Green, S.L., Galligan, D.T., Stanley, E.K., Osborne, J., 1993. Diagnosis and prediction of cervical vertebral malformation in thoroughbred foals based on semi-quantitative radiographic indicators. Equine Veterinary Journal 25, 435-440.

Mayhew, I.G., Washbourne, J.R., 1996. Magnetic motor evoked potentials in ponies. Journal of Veterinary Internal Medicine 10, 326-329.

Mayhew, I.G. Large animal neurology. 2nd Edition. Iowa: Wiley-Blackwell; 2008

Mills, K.R., Murray, N.M., 1986. Electrical stimulation over the human vertebral column: which neural elements are excited? Electroencephalography and Clinical Neurophysiology 63, 582-589.

Mitchell, C.W., Nykamp, S.G., Foster, R., Cruz, R., Montieth, G., 2012. The Use of Magnetic Resonance Imaging in Evaluating Horses with Spinal Ataxia. Veterinary Radiology and Ultrasound 53, 613-620.

Moore, B.R., Holbrook, T.C., Stefanacci, J.D., Reed, S.M., Tate, L.P., Menard, M.C., 1992. Contrast-enhanced computed tomography and myelography in six horses with cervical stenotic myelopathy. Equine Veterinary Journal 24, 197-202.

Moore, B.R., Reed, S.M., Biller, D.S., Kohn, C.W., Weisbrode, S.E., 1994. Assessment of vertebral canal diameter and bony malformations of the cervical part of the spine in horses with cervical stenotic myelopathy. American Journal of Veterinary Research 55, 5-13.

Moore, B.R., Reed, S.M., Robertson, J.T., 1993. Surgical treatment of cervical stenotic myelopathy in horses: 73 cases (1983-1992). Journal of the American Veterinary Medical Association 203, 108-112.

Mullen, K.R., Furness, M.C., Johnson, A.L., Norman, T.E., Hart, K.A., Burton, A.J., Bicahlo, R.C., Ainsworth, D.M., Thompson, M.S., Scrivani, P.V., 2015. Adverse reactions in horses that underwent general anesthesia and cervical myelography. Journal of Veterinary Internal Medicine 29, 954-960.

Nakamae, T., Tanaka, N., Nakanishi, K., Fujimoto, Y., Sasaki, H., Kamei, N., Hamasaki, T., Yamada, K., Yamamoto, R., Izumi, B., Ochi, M., 2010. Quantitative assessment of

myelopathy patients using motor evoked potentials produced by transcranial magnetic stimulation. European Spine Journal 19, 685-690.

Nakanishi, K., Tanaka, N., Kamei, N., Ohta, R., Fujioka, Y., Hiramatsu, T., Ujigo, S., Ochi, M., 2014. Electrophysiological evidence of functional improvement in the corticospinal tract after laminoplasty in patients with cervical compressive myelopathy. Journal of Neurosurgery Spine 21, 210-216.

Nappert, G., Vrins, A., Breton, L., Beauregard, M., 1989. A Retrospective Study of 19 Ataxic Horses. Canadian Veterinary Journal -Revue Veterinaire Canadienne 30, 802-806.

Nardone, R., Holler, Y., Brigo, F., Frey, V.N., Lochner, P., Leis, S., Golaszewski, S., Trinka, E., 2016. The contribution of neurophysiology in the diagnosis and management of cervical spondylotic myelopathy: a review. Spinal cord 54, 756-766.

Nardone, R., Holler, Y., Thomschewski, A., Holler, P., Bergmann, J., Golaszewski, S., Brigo, F., Trinka, E., 2014. Central motor conduction studies in patients with spinal cord disorders: a review. Spinal cord 52, 420-427.

Nixon, A.J., Stashak, T.S., 1983. Dorsal Laminectomy in the Horse .1. Review of the Literature and Description of a New Procedure. Veterinary Surgery 12, 172-176.

Nollet, H., Deprez, P., Van Ham, L., Verschooten, F., Vanderstraeten, G., 2002. The use of magnetic motor evoked potentials in horses with cervical spinal cord disease. Equine Veterinary Journal 34, 156-163.

Nollet, H., Deprez, R., Van Ham, L., Dewulf, J., Decleir, A., Vanderstraeten, G., 2004. Transcranial magnetic stimulation: normal values of magnetic motor evoked potentials in 84 normal horses and influence of height, weight, age and sex. Equine Veterinary Journal 36, 51-57.

Nollet, H., Van Ham, L., Dewulf, J., Vanderstraeten, G., Deprez, P., 2003a. Standardization of transcranial magnetic stimulation in the horse. Veterinary Journal 166, 244-250.

Nollet, H., Van Ham, L., Gasthuys, F., Dewulf, J., Vanderstraeten, G., Deprez, P., 2003b. Influence of detomidine and buprenorphine on motor-evoked potentials in horses. Veterinary Record 152, 534-537.

Nollet, H., Van Ham, L., Verschooten, F., Vanderstraeten, G., Deprez, P., 2003c. Use of magnetic motor-evoked potentials in horses with bilateral hind limb ataxia. American Journal of Veterinary Research 64, 1382-1386.

Nollet, H., Vanschandevijl, K., Van Ham, L., Vanderstraeten, G., Deprez, P., 2005. Role of transcranial magnetic stimulation in differentiating motor nervous tract disorders from other causes of recumbency in four horses and one donkey. Veterinary Record 157, 656-658.

Nout, Y.S., Reed, S.M., 2003. Cervical vertebral stenotic myelopathy. Equine Veterinary Education 15, 212-223.

Olsen, E., Dunkel, B., Barker, W.H., Finding, E.J., Perkins, J.D., Witte, T.H., Yates, L.J., Andersen, P.H., Baiker, K., Piercy, R.J., 2014. Rater agreement on gait assessment during neurologic examination of horses. Journal of Veterinary Internal Medicine 28, 630-638.

Oswald, J., Love, S., Parkin, T.D., Hughes, K.J., 2010. Prevalence of cervical vertebral stenotic myelopathy in a population of thoroughbred horses. Veterinary Record 166, 82-83.

Papageorges, M., Gavin, P.R., Sande, R.D., Barbee, D.D., Grant, B.D., 1987. Radiographic and Myelographic Examination of the Cervical Vertebral Column in 306 Ataxic Horses. Veterinary Radiology 28, 53-59.

Poma, R., Parent, J.M., Holmberg, D.L., Partlow, G.D., Monteith, G., Sylvestre, A.M., 2002. Correlation between severity of clinical signs and motor evoked potentials after transcranial magnetic stimulation in large-breed dogs with cervical spinal cord disease. Journal of the American Veterinary Medical Association 221, 60-64.

Powers, B.E., Stashak, T.S., Nixon, A.J., Yovich, J.V., Norrdin, R.W., 1986. Pathology of the Vertebral Column of Horses with Cervical Static Stenosis. Veterinary pathology 23, 392-399.

Prange, T., Carr, E.A., Stick, J.A., Garcia-Pereira, F.L., Patterson, J.S., Derksen, F.J., 2012. Cervical vertebral canal endoscopy in a horse with cervical vertebral stenotic myelopathy. Equine Veterinary Journal 44, 116-119.

Prange, T., Derksen, F.J., Stick, J.A., Garcia-Pereira, F.L., Carr, E.A., 2011. Cervical vertebral canal endoscopy in the horse: intra- and post operative observations. Equine Veterinary Journal 43, 404-411.

Reardon, R., Kummer, M., Lischer, C., 2009. Ventral Locking Compression Plate for Treatment of Cervical Stenotic Myelopathy in a 3-Month-Old Warmblood Foal. Veterinary Surgery 38, 537-542.

Reardon, R.J.M., Bailey, R., Walmsley, J.P., Heller, J., Lischer, C., 2010. An In Vitro Biomechanical Comparison of a Locking Compression Plate Fixation and Kerf Cut Cylinder Fixation for Ventral Arthrodesis of the Fourth and the Fifth Equine Cervical Vertebrae. Veterinary Surgery 39, 980-990.

Rose, P.L., Abutarbush, S.M., Duckett, W., 2007. Standing myelography in the horse using a nonionic contrast agent. Veterinary Radiology and Ultrasound 48, 535-538.

Saville, W.J.A., Reed, S.M., Dubey, J.P., Granstrom, D.E., Morley, P.S., Hinchcliff, K.W., Kohn, C.W., Wittum, T.E., Workman, J.D., 2017. Interobserver Variation in the Diagnosis of Neurologic Abnormalities in the Horse. Journal of Veterinary Internal Medicine 31, 1871-1876.

Schmidburg, I., Pagger, H., Zsoldos, R.R., Mehnen, J., Peham, C., Licka, T.F., 2012. Movement associated reduction of spatial capacity of the equine cervical vertebral canal. Veterinary Journal 192, 525-528.

Sleutjens, J., Cooley, A.J., Sampson, S.N., Wijnberg, I.D., Back, W., van der Kolk, J.H., Swiderski, C.E., 2014. The equine cervical spine: comparing MRI and contrast-enhanced CT images with anatomic slices in the sagittal, dorsal, and transverse plane. The Veterinary quarterly 34, 74-84.

Smyth, G.B., 1993. Use of ventral cervical stabilization for treatment of a suspected articular facet fracture in a horse. Journal of the American Veterinary Medical Association 202, 771-772.

Stewart, R.H., Reed, S.M., Weisbrode, S.E., 1991. Frequency and severity of osteochondrosis in horses with cervical stenotic myelopathy. American Journal of Veterinary Research 52, 873-879.

Sylvestre, A.M., Cockshutt, J.R., Parent, J.M., Brooke, J.D., Holmberg, D.L., Partlow, G.D., 1993. Magnetic motor evoked potentials for assessing spinal cord integrity in dogs with intervertebral disc disease. Veterinary Surgery 22, 5-10.

Szklarz, M., Skalec, A., Kirstein, K., Janeczek, M., Kasparek, M., Kasparek, A., Waselau, M., 2018. Management of equine ataxia caused by cervical vertebral stenotic myelopathy: A European perspective 2010-2015. Equine Veterinary Education 30, 370-376.

Travlos, A., Pant, B., Eisen, A., 1992. Transcranial magnetic stimulation for detection of preclinical cervical spondylotic myelopathy. Archives of physical medicine and rehabilitation 73, 442-446.

Trostle, S.S., Dubielzig, R.R., Beck, K.A., 1993. Examination of Frozen Cross-Sections of Cervical Spinal Intersegments in 9 Horses with Cervical Vertebral Malformation - Lesions Associated with Spinal-Cord Compression. Journal of Veterinary Diagnostic Investigation 5, 423-431.

Tyler, C.M., Davis, R.E., Begg, A.P., Hutchins, D.R., Hodgson, D.R., 1993. A survey of neurological diseases in horses. Australian Veterinary Journal 70, 445-449.

van Biervliet, J., Scrivani, P.V., Divers, T.J., Erb, H.N., de Lahunta, A., Nixon, A., 2004. Evaluation of decision criteria for detection of spinal cord compression based on cervical myelography in horses: 38 cases (1981-2001). Equine Veterinary Journal 36, 14-20.

Van Ham, L.M., Nijs, J., Mattheeuws, D.R., Vanderstraeten, G.G., 1996a. Sufentanil and nitrous oxide anaesthesia for the recording of transcranial magnetic motor evoked potentials in dogs. Veterinary Record 138, 642-645.

Van Ham, L.M., Nijs, J., Vanderstraeten, G.G., Mattheeuws, D.R., 1996b. Comparison of two techniques of narcotic-induced anesthesia for use during recording of magnetic motor evoked potentials in dogs. American Journal of Veterinary Research 57, 142-146.

Van Ham, L.M., Vanderstraeten, G.G.W., Mattheeuws, D.R.G., Nijs, J., 1994. Transcranial Magnetic Motor Evoked-Potentials in Sedated Dogs. Progress in veterinary neurology 5, 147-154.

Veraa, S., Bergmann, W., van den Belt, A.J., Wijnberg, I., Back, W., 2016. Ex Vivo Computed Tomographic Evaluation of Morphology Variations in Equine Cervical Vertebrae.

Veterinary radiology & ultrasound : the official journal of the American College of Veterinary Radiology and the International Veterinary Radiology Association 57, 482-488.

Walmsley, J.R., 2005. Surgical treatment of cervical spinal cord compression in horses: a European experience. Equine Veterinary Education 17, 39-43.

Wassermann, E.M., 1998. Risk and safety of repetitive transcranial magnetic stimulation: report and suggested guidelines from the International Workshop on the Safety of Repetitive Transcranial Magnetic Stimulation, June 5-7, 1996. Electroencephalography and Clinical Neurophysiology 108, 1-16.

Widmer, W.R., Blevins, W.E., Jakovljevic, S., Levy, M., Teclaw, R.F., Han, C.M., Hurd, C.D., 1998. A prospective clinical trial comparing metrizamide and iohexol for equine myelography. Veterinary Radiology and Ultrasound 39, 106-109.

Wijnberg, I.D., 2005. A review of the use of electromyography in equine neurological diseases. Equine Veterinary Education 17, 123-127.

Wijnberg, I.D., Back, W., de Jong, M., Zuidhof, M.C., van den Belt, A.J., van der Kolk, J.H., 2004. The role of electromyography in clinical diagnosis of neuromuscular locomotor problems in the horse. Equine Veterinary Journal 36, 718-722.

Yamada, K., Sato, F., Hada, T., Horiuchi, N., Ikeda, H., Nishihara, K., Sasaki, N., Kobayashi, Y., Nambo, Y., 2016. Quantitative evaluation of cervical cord compression by computed tomographic myelography in Thoroughbred foals.Journal of Equine Science 27, 143-148.

CHAPTER 2

SCIENTIFIC AIMS

Despite continuous scientific progress, evaluation of horses and cattle with neurological complaints strongly depends on the subjective and rather limited neurological examination. The best method to confirm spinal cord compression is currently myelography, but the sensitivity is low and the procedure is not without risks. Medical imaging techniques are improving and will probably become (more widely) available in the future, but still, they are expensive, often require general anaesthesia and do not provide information about the spinal cord function. The risk of over-interpretation of non-functional lesions on these detailed images is real.

TMS can offer an alternative, as it is a cheap, non-invasive functionality test. Despite its use to diagnose spinal cord disease for several years, it has not developed towards a mainstream test, because only gross localization of lesions is possible in horses and the diagnostic accuracy of TMS-MMEP is unknown.

Therefore, the overall objective of the present thesis was to further develop and validate TMS as a quick and cheap diagnostic test for spinal cord dysfunction in large animals. The specific objectives were:

1. To explore and determine reference values for TMS-MMEP in other large animals, such as ruminants (Chapter 3)
2. To improve the technique of TMS-MMEP in horses by
    a. determination of reference values for cervical spinal muscles with the goal to enable more specific localization of spinal cord lesions in the future (Chapter 4)
    b. using non-invasive surface electrode recording instead of intramuscular needle electrode recording (Chapter 5)
3. To validate TMS as a diagnostic test in horses by
    a. Determining clear cut-off values for latency based on controls and confirmed cases (Chapter 6)
    b. Using a Bayesian latent class analysis, comparing diagnostic accuracy of clinical examination, cervical radiographs and TMS in horses admitted under suspicion of a neurological gait abnormality (Chapter 7)

CHAPTER 3

MOTOR EVOKED POTENTIALS IN STANDING AND RECUMBENT CALVES INDUCED BY MAGNETIC STIMULATION AT THE FORAMEN MAGNUM

MOTOR EVOKED POTENTIALS IN STANDING AND RECUMBENT CALVES INDUCED BY MAGNETIC STIMULATION AT THE FORAMEN MAGNUM

J. Rijckaert[1], B. Pardon[1], K. Verryken[1], L. Van Ham[2], G. van Loon[1], P. Deprez[1]

[1] Department of Large Animal Internal Medicine, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, Merelbeke 9820, Belgium

[1] Department of Small Animals, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, Merelbeke 9820, Belgium

Adapted from: Rijckaert, J., Pardon, B., Verryken, K., Van Ham, L., van Loon, G., Deprez, P., 2016. Motor evoked potentials in standing and recumbent calves induced by magnetic stimulation at the foramen magnum. Veterinary Journal 216, 178-182.

ABSTRACT

The aims of this study were to determine reference values for magnetic motor evoked potentials (MMEPs) in calves and the influence of position during examination (standing or lateral recumbency). Reference values were determined using 41 healthy Holstein Friesian bull calves aged 1-10 months; standing and lateral recumbency were examined in 11 calves. Maximal magnetic stimulation was performed at the level of the foramen magnum with a magnetic field of 4 T at the coil surface.

In standing position, clear, reproducible MMEPs were obtained in all calves. Onset latency (LAT) and amplitude (AMPL) (mean ± standard deviation) were 34.4 ± 3.1 ms and 3.7 ± 1.7 mV in the thoracic limbs. These values were significantly different from those in the hind limbs (LAT 44.6 ± 3.0 ms; AMPL 3.3 ± 1.7 mV). AMPL significantly increased with body length. Age, body weight, height at the withers and rectal temperature had no significant relationship with LAT or AMPL. No left to right differences were noted. In the lateral position, only 64% of the calves showed responses in the four limbs. LAT and AMPL were 29.7 ± 4.7 ms and 3.0 ± 1.8 mV in the thoracic limbs, and were significantly different from these parameters in the hind limbs (47.0 ± 7.4 ms and 2.1 ± 2.1 mV, respectively).

In conclusion, magnetic stimulation is possible in calves at the foramen magnum level. In lateral decubitus, the MMEPs are more difficult to register than in standing calves.

Key words*:* Calf; Electromyograph; Magnetic stimulation; Neurological test

INTRODUCTION

In calves and adult cattle, prolonged recumbency is a frequent problem. The list of differential diagnoses for prolonged recumbency or an inability to stand includes numerous traumatic, metabolic, infectious, toxic, nutritional and neoplastic causes (Sherman, 1987). In dairy cows, periparturient recumbency is most frequently related to hypocalcaemia (Gelfert et al., 2007), but traumatic lesions, such as fractures or nerve compression, are frequent complications. In calves, spinal cord compression can be due to epidural haematomas or abscesses (Zani et al., 2008), osteomyelitis (Healy et al., 1997) or cervical fractures. However, diagnosis is often difficult due to the limitations of the neurological examination in cattle. In contrast to small animals (Parent, 2010), extended neurological examinations and magnetic resonance imaging or computed tomography often are not feasible in cattle.

Transcranial magnetic stimulation is a rapid and relatively inexpensive modality that excites motor neurones through the cranium. This results in an impulse that is conducted through the motor pathways to the muscles, where magnetic motor evoked potentials (MMEPs) can be recorded. These potentials and their specific characteristics have been used to evaluate nerve conduction in humans (Chen et al., 2008; Nardone et al., 2014; Rossini et al., 2015), dogs (Van Ham et al., 1996; da Costa et al., 2006; De Decker et al., 2011) and horses (Mayhew and Washbourne, 1996; Nollet et al., 2002, 2003b, 2004). The aims of the present study were to establish reference values for onset latency (LAT) and amplitude (AMPL) of MMEPs in calves and to assess whether these values are influenced by standing or lateral recumbency.

MATERIALS AND METHODS

ANIMALS

Forty-one animals used for recording normal MMEPs were healthy Holstein Friesian (HF) bull calves aged 1-10 (median 2) months. At the time of the first test, the median body weight was 64 (range 49-230) kg, the median height at the withers was 85 (range 79-113) cm and the median length from the occiput to the base of the tail was 104 (range 88-150) cm. Nine calves were tested a second time within a period of 1 month after the first examination. These results were included in the calculations of reference values. In the second part of the study, 11/41 calves were selected at random to study the effect of position. In this subgroup, the median age was 2 (range 1-3) months, the median weight was 67 (range 49-80) kg, the median height at the withers was 85 (range 80-93) cm and the median length was 98 (range 88-120) cm. None of the calves showed abnormalities on neurological examination. Recommendations of the Quality Assurance and Laboratory Standards Committee (QALS; Geffré et al., 2009) were followed to determine reference values. All procedures were approved by the ethical committee of the Faculty of Veterinary Medicine, Ghent University (EC 2012039, date of approval 15 April 2012; EC 2013022, date of approval 6 March 2013).

MAGNETIC MOTOR EVOKED POTENTIALS

Reference values and repeatability - Rectal temperatures of each calf were determined at the start of each procedure. Stimulation was performed in unsedated calves. Needle electrodes were placed as for horses (Nollet et al. 2004). The active needle electrode (25 mm monopolar, disposable, insulated, stainless steel, TECA Corporation) was placed in the middle of the tibialis cranialis muscle in the pelvic limbs and the extensor carpi radialis muscle in the thoracic limbs. The reference electrode was placed subcutaneously at the central part of the corresponding extensor digitalis lateralis muscle. The ground electrode was attached in the inguinal region while testing the hind limbs and in the axillary region while testing the thoracic limbs. The calves were stimulated with a magnetic stimulator (Magstim 200, The Magstim Company) using a round 70 mm coil, generating a maximal magnetic field of 4 Tesla at the coil surface. The coil was centred on the skin above the foramen magnum and a maximal stimulus intensity (100%) was applied. The trigger output from the magnetic stimulator was connected to the

input port of the standard electromyograph (EMG; Medelec Sapphire, Medelec) used to record the muscle response from the needles in the limb. Through this connection, the magnetic stimulation and MMEP recording were synchronised.

To obtain the MMEP with the shortest LAT and largest AMPL, the head of the calf was slightly flexed, making the angle between the dorsal neck and the frontal surface of the head ~90° (Fig. 1). Four sequential muscle responses were recorded from each limb, with a rest period of 10-15 seconds between each stimulation. The sequence of recording in the different limbs was randomised. LAT (ms) was defined as the time between the onset of stimulation and the onset of contraction, indicated by the first deflections from the baseline. AMPL (mV) was defined as the difference between the two largest peaks of opposite polarity. All stimulations and the positioning of markers for LAT and AMP on the EMG screen were performed by the same operator.

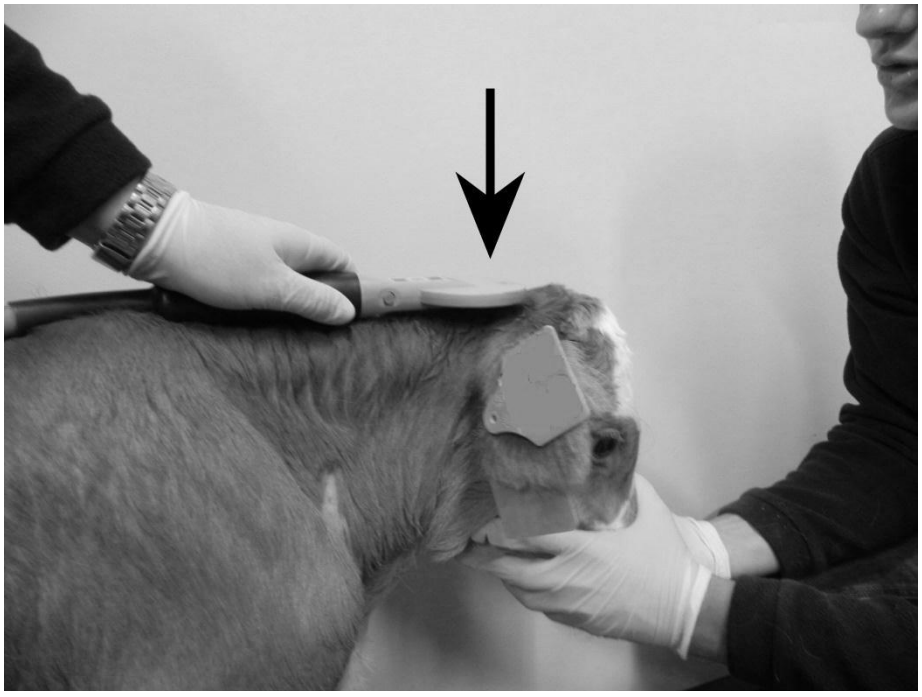


Figure. 1. Position of head and neck of a calf during magnetic stimulation. The arrow indicates the centre of the coil, positioned over the foramen magnum.

EFFECT OF POSITION ON MAGNETIC MOTOR EVOKED POTENTIALS

To determine the effect of position (standing or recumbent) on LAT and AMPL, 11 calves were stimulated twice at an interval of 4 h, once in a standing and once in a randomly selected left or right lateral position. The procedure applied in the standing position was analogous to the first experiment. For the lateral position, the calves were randomly restrained on a mattress. For stimulation, the same coil position and stimulus intensity were used as in the standing position.

DATA MANAGEMENT AND STATISTICAL ANALYSIS

The continuous outcome variables LAT and AMPL were checked for a normal distribution (Kolmogorov Smirnov test and inspection of Q-Q plots and histograms). AMPL showed a right skewed distribution and was Ln+1 transformed to obtain a normal distribution. To determine repeatability of MMEPs, coefficients of variation were determined at the level of the limb (four measurements per limb) and the level of the calf (mean of the four measurements per limb): CV (%) = (SD/mean) x 100. To determine the 90% reference intervals (upper and lower bound) for LAT and AMPL in healthy standing calves, an Excel add-in was used (Reference Value Advisor; Geffré et al., 2011). This programme calculates untransformed, box transformed and non-parametric reference intervals, and indicates which intervals comply best with the data set.

To determine factors which are significantly associated with LAT and AMPL in healthy calves, the mean of the four measurements on each limb was used as the outcome variable. A mixed model was built with calf and limb added as random effects to account for clustering of measurements within a calf and within a limb (PROC MIXED). Predictors added to the model were limb (thoracic/pelvic and left/right), testing number (first, second, third or fourth stimulation) and physiological parameters, i.e. age (months), length (cm), body weight (kg), height at the withers (cm) and rectal temperature (°C). Firstly, all parameters were tested univariably. All parameters with $P < 0.20$ were retained in the final multivariable model, which was built stepwise backwards, to exclude non-significant factors. When predictor variables were highly correlated (> 0.60), only the most significant variable was added to the model. All biologically relevant interactions of significant main effects were tested. For all significant

categorical variables, pairwise comparisons were made using post-hoc tests with a Bonferroni correction to adjust for multiple comparisons.

To determine the effect of position (standing or lateral recumbency) on LAT and AMPL, a linear mixed model was built (PROC MIXED). The recording before (standing) and after (recumbent) in each animal was added as a repeated effect. Calf and limb were added as random effects to account for clustering of measurements within a limb within a calf. Body weight was added as a continuous variable in each model. For all models, significance was set at $P < 0.05$. All analyses were performed in SAS vs. 9.3 (SAS Institute).

RESULTS

RECORDING OF MAGNETIC MOTOR EVOKED POTENTIALS

In 800 stimulations in standing calves, 730 appropriate potentials were recorded in which AMPL could be determined with confidence (Figs. 2A and B). MMEPs were recordable at least 3/4 times in every limb of 36/41 calves, with 100% response in the thoracic limbs. In 3/41 (7.3%) calves, none of the hind limbs showed a response to stimulation and in another 2/41 (4.9%) there were no measureable reactions in one hind limb. Means and reference values for LAT and AMPL are shown in Table 1 and the repeatability of the technique at limb and calf level are shown in Table 2. The CV was larger for AMPL than for LAT, both at limb and at calf level.

For LAT, 4.6%, 77.9% and 17.4% of the variation was present at calf, limb and measurement level, respectively. Factors univariably associated with LAT were length ($P < 0.001$), weight ($P < 0.001$), thoracic or pelvic limb ($P < 0.001$) and temperature ($P < 0.05$). In the final multivariable model for LAT, only the factor thoracic/pelvic limb remained significant ($P < 0.001$), with a mean difference of 11.2 ± 0.5.

For AMPL, 26.5%, 24.5% and 48.9% of the variation occurred at calf, limb and measurement level, respectively. Factors univariably associated with AMPL were length ($P < 0.01$), weight ($P < 0.001$), height ($P < 0.05$) and thoracic/pelvic limb ($P < 0.001$). Since the first three parameters were highly correlated, the most significant (weight) was used in the multivariable model. This final multivariable model consisted of weight ($P < 0.001$) and thoracic/pelvic limb ($P < 0.001$).

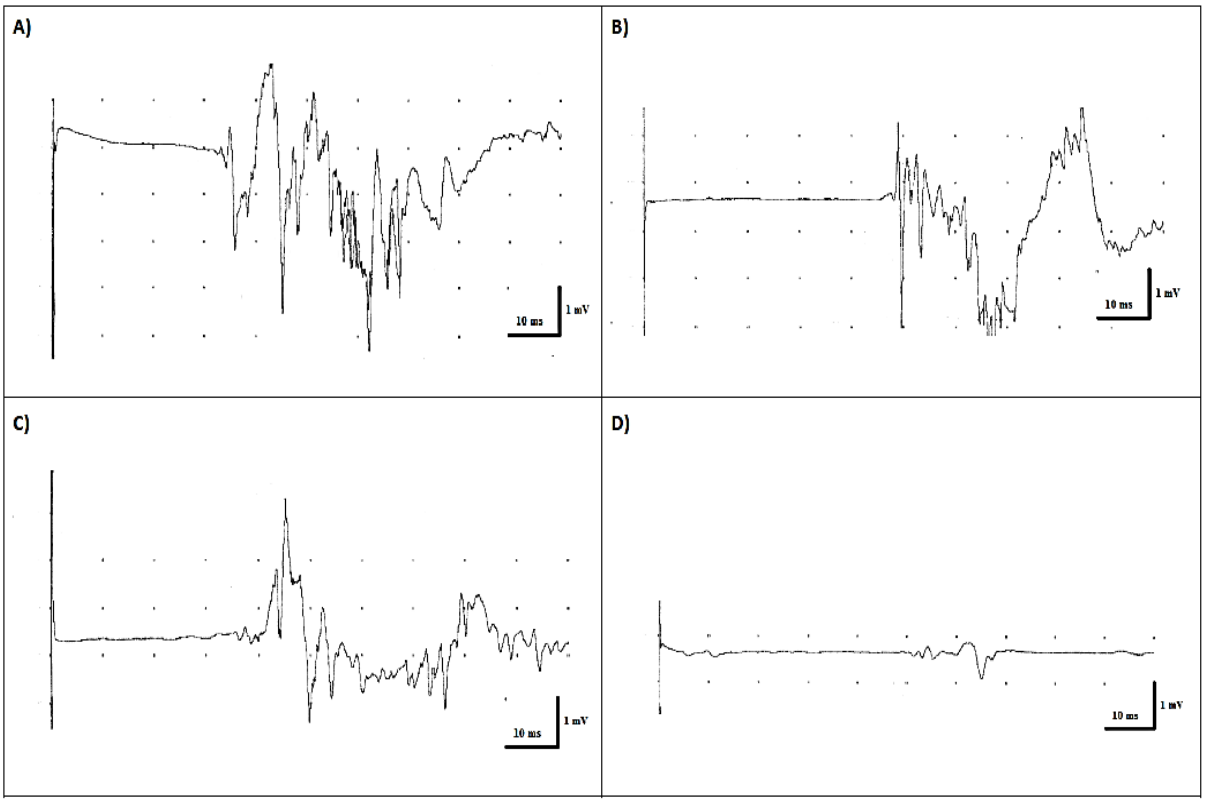


Fig. 2. Example of magnetic motor evoked potentials (MMEPs) of a 3-month-old calf. (A) Left thoracic limb, standing position. (B) Left pelvic limb, standing position. (C) Left thoracic limb, left lateral position. (D) Left pelvic limb, lateral position.

Table 1

Mean observed values, standard deviations (SD), minimum (Min) and maximum (Max) values, and calculated reference intervals (RI) for latency and amplitude in thoracic and pelvic limbs in 41 healthy standing calves.

	Limb	*n*	Mean	SD	Min-Max	RI
Latency (ms)	Thoracic	400	34.4	3.1	13.9-54.7	24.3-43.9
	Pelvic	348	44.6	3.0	31.4-65.3	35.7-53.8
Amplitude (mV)	Thoracic	398	3.7	1.7	0.2-13.9	1.4-7.7
	Pelvic	332	3.3	1.7	0.2-18.1	0.6-7.9

n, number of magnetic motor evoked potentials recorded.

Table 2

Repeatability of MMEPs in calves expressed as coefficient of variation (CV), mean values, standard deviations (SD), and minimum (Min) and maximum (Max) values at limb and calf level.

			Latency				Amplitude			
		n	Mean CV (%)	SD	Min	Max	Mean CV (%)	SD	Min	Max
Limb level	Thoracic limb	400	10.0	4.6	2.2	25.7	52.0	15.6	16.9	97.9
	Pelvic limb	348	7.3	5.0	1.9	26.0	54.6	17.9	15.9	105.2
Calf level	Thoracic limb	398	11.2	7.0	0.3	37.6	45.2	25.2	6.2	200.0
	Pelvic limb	332	8.9	6.0	1.4	35.1	49.5	24.7	10.8	126.4

Each limb was measured four times.

n, number of magnetic motor evoked potentials recorded.

In response to stimulation, the calves also showed a startle reflex. This reflex is a brief generalised muscle contraction evoked by an unexpected stimulus, which varies with the mental state of the animal and habituation or attenuation (Grillon and Baas, 2003). The startle reflex was most distinct after the first stimulations and diminished progressively with the increasing number of stimulations. Some of these muscle contractions were also recorded by the electromyograph. Distinction between this irregular startle response and the MMEP was made based on the high reproducibility of the MMEPs.

INFLUENCE OF POSITION ON MAGNETIC MOTOR EVOKED POTENTIALS

In lateral recumbency, LAT could be measured in all calves at least one time in the thoracic limbs, but only 7/11 (63.6%) calves showed at least one measurable response in each hind limb. In two calves, there was no measurable response in any hind limb, while in another two calves there were no detectable MMEPs in the dependent limb. Of all stimulations, 6/176 (3.4%) recordings in the thoracic limbs and 98/176 (55.7%) recordings in the hind limbs were not measurable. In total, 280 measurements from 11 calves were available for statistical modelling (Table 3; Figs. 2C and D). Position affected LAT (significant interaction; $P < 0.001$); in recumbent calves, LAT decreased in the thoracic limbs, but increased in the hind limbs. For AMPL, 254 measurements in 11 calves were usable. There was no significant influence of position in the thoracic limb; however, compared to the standing position, significantly lower AMPL values were present in the pelvic limbs ($P < 0.001$; Fig. 3).

Table 3

Mean values, standard deviations (SD), minimum (Min) and maximum (Max) values, and calculated reference intervals (RI) for latency and amplitude in the thoracic and the pelvic limbs in calves in lateral position.

		n	Mean	SD	Min-Max	RI	Mean CV (%)
Latency (ms)	Thoracic limb	85	29.7	4.7	19.7-54.1	20.5-38.9	17%
	Pelvic limb	85	47.0	7.4	36.4-69.7	32.6-61.4	16%
Amplitude (mV)	Thoracic limb	63	3.0	1.8	0.6-11.9	0-6.6	59%
	Pelvic limb	39	2.1	2.1	0.3-6.9	0-6.2	97%

CV, coefficient of variation (%).

n, number of magnetic motor evoked potentials recorded

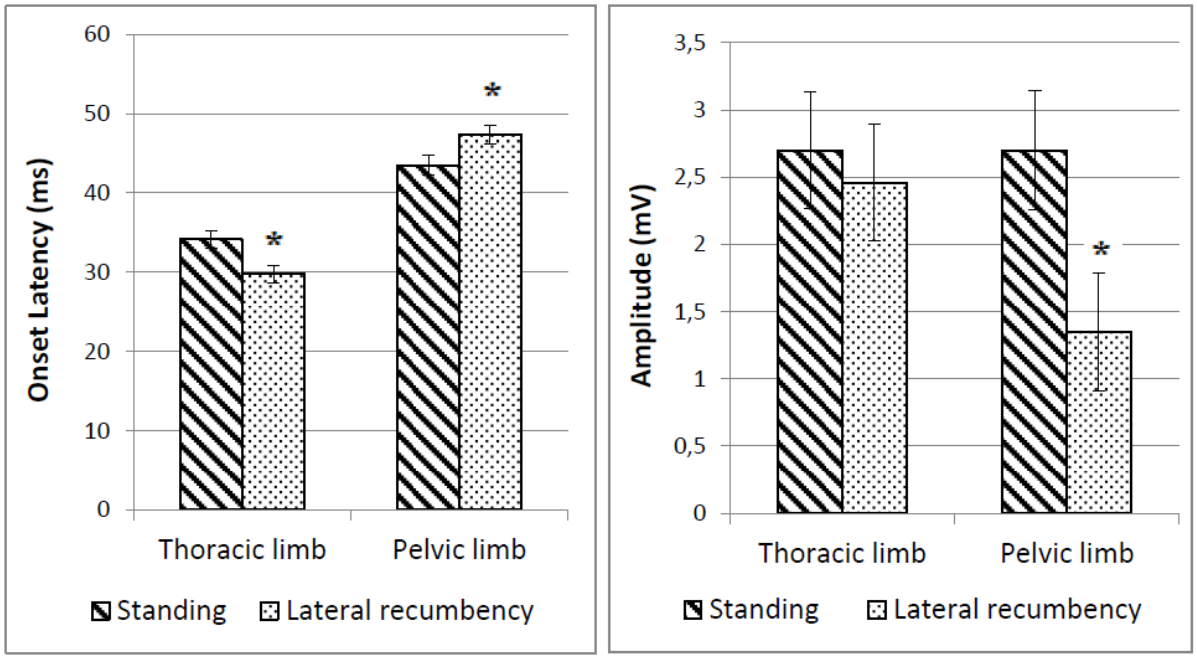


Fig. 3. Least square means ± standard deviation of a multivariable mixed model for onset latency (left) and amplitude (right) in 290 and 254 magnetic motor evoked potentials (MMEPs), respectively, in 11 calves in standing and lateral recumbent position. * $P < 0.001$ between positions.

DISCUSSION

Recording of MMEPs is possible in bull calves aged 1-10 months. However, the stimulation site used here differs from that used in other species. In human beings and dogs, the optimal stimulation site to evoke MMEPs in the limbs is centrally on the vertex (Hess and Ludin, 1988; Van Ham et al., 1994), while in horses it is centrally on the forehead (Nollet et al., 2003a). However, our unpublished transcranial stimulation pilot studies in calves resulted in absent or small, variable MMEPs. This probably because the distance and resistance from skin to cortex in calves is larger at the vertex and forehead compared to human beings, dogs and horses, because of the presence of the frontal sinus and horns. Therefore, the calves were magnetically stimulated at the level of the foramen magnum, as described in human beings (Ugawa et al., 1997). Since the foramen magnum is large in calves (about 4 cm in diameter) and the distance from the skin to the spinal cord at this point is only 2-3 cm, the magnetic field can reach the spinal cord (the motor cortex is probably not involved). The part of the magnetic field that reaches the motor tracts more readily will be increased by flexion of the neck, resulting in a maximised foramen magnum and thereby less deflection of the magnetic field. Some contraindications are known in human medicine (Rossi et al., 2009); in calves, a history of seizure or suspicion of vertebral fracture are most important, since stimulation could worsen neurological signs.

LAT is a valuable parameter in calves, with a small CV at limb and at calf level, but two differences compared to other species were found. Firstly, there was no significant effect of height, weight and length, such effects have been reported in previous studies in other species (Furby et al., 1992; Nollet et al., 2004; Matamala et al., 2013). This difference may be due to the lower power of the present study or the more limited variation in physical characteristics in our subjects. Secondly, LAT is longer in calves than in horses (Nollet et al., 2004). This can be explained by a difference in nerve conduction velocity in young cattle compared to adult horses. However, peripheral nerve conduction velocity in calves is high compared to other species (Schenk et al., 2014), suggesting that the difference in LAT is due to slower conduction through the central nervous system. Alternatively, the longer LAT in calves could be due to a different type of generated response, i.e. an indirect response (I-wave) instead of a direct response (D-wave) (Ugawa et al., 1997). The I-wave is a trans-synaptical, cortical reflex and

can be provoked at lower stimulus intensities compared to the faster D-wave (Cassim and Houdayer, 2006). Low magnetic stimulus intensity is suspected in foramen magnum stimulation; it is possible that only a proportion of the magnetic field reaches the spinal cord, since the bony structures surrounding the foramen magnum can deflect the magnetic field (Matsumoto et al., 2013).

AMPL values were smaller in calves than in dogs (Van Ham et al., 1994) and horses (Nollet et al., 2004), in which the parameter increases with weight, has a large CV and is more difficult to determine than LAT. In responses with very low AMPL values (less than 0.3 mV), also LAT is difficult to determine and more variation in LAT is possible. Therefore, these ambiguous responses might be arbitrary muscle reactions on stimulation (startle reflex) instead of real MMEPs. The increase in AMPL with weight might be a natural consequence of muscle hypertrophy in growing calves (Preston and Shapiro, 2002). Furthermore, AMPL and its repeatability can be influenced by differences in pre-stimulus muscle tension, based on the facilitation principle, in which voluntary contraction of the target muscles increases amplitude (Mills et al., 1987; Di Lazzaro et al., 1998), submaximal stimulation or suboptimal coil position (Nollet et al., 2003a) and the use of needle electrodes instead of surface electrodes (Preece et al., 1994; van Dijk et al., 1995). Therefore, optimisation of the magnetic stimulation technique in calves might offer opportunities to improve AMPL determination and repeatability.

The effect of posture appears to be important. The values for LAT and AMPL were substantially different from the reference values in standing calves and often no measurable potentials could be registered when the calves were restrained in the lateral position. Thus, there are limitations for using MMEPs in recumbent calves. In human beings, Abraham et al. (2013) described a reduction in AMPL of MMEPs as a result of neck flexion. However, in our calves the degree of neck flexion in lateral position was similar to the flexion in standing position. A possible explanation for the different values in lateral position could be the voluntary contraction theory (facilitation principle) because muscle tension will be different in lateral than in standing position. The extensor carpi radialis muscle has a more pronounced antigravity activity than the tibialis cranialis muscle and should therefore show shorter LAT and bigger AMPL in standing position compared to the lateral position. AMPL was larger but

also LAT increased in the thoracic limbs in standing position while it decreased in the pelvic limbs.

CONCLUSIONS

Magnetic stimulation to generate in MMEPs in calves is possible at the level of the foramen magnum. LAT is a valuable parameter with a small CV. AMPL is not reliable because of the large variation in resulting waveforms. In standing position, the test delivers clear, highly reproducible results. In lateral recumbency, MMEPs are more difficult to obtain and significantly altered in both thoracic (LAT) and hind limbs (LAT and AMPL).

REFERENCES

Abraham, A., Gotkine, M., Drory, V.E., Blumen, S.C., 2013. Effect of neck flexion on somatosensory and motor evoked potentials in Hirayama disease. Journal of Neurological Science 334, 102-105.

Cassim, F., Houdayer, E., 2006. Neurophysiology of myoclonus. Clinical Neurophysiology 36, 281-291.

Chen, R., Cros, D., Curra, A., Di Lazzaro, V., Lefaucheur, J.P., Magistris, M.R., Mills, K., Rosler, K.M., Triggs, W.J., Ugawa, Y., et al., 2008. The clinical diagnostic utility of transcranial magnetic stimulation: Report of an IFCN committee. Clinical Neurophysiology 119, 504-532.

da Costa, R.C., Poma, R., Parent, J.M., Partlow, G., Monteith, G., 2006. Correlation of motor evoked potentials with magnetic resonance imaging and neurologic findings in Doberman Pinschers with and without signs of cervical spondylomyelopathy. Journal of the American Veterinary Medical Association 67, 1613-1620.

De Decker, S., Van Soens, I., Duchateau, L., Gielen, I.M., van Bree, H.J., Binst, D.H., Waelbers, T., Van Ham, L.M., 2011. Transcranial magnetic stimulation in Doberman Pinschers with clinically relevant and clinically irrelevant spinal cord compression on magnetic resonance imaging. Journal of the American Veterinary Medical Association 238, 81-88.

Di Lazzaro, V., Restuccia, D., Oliviero, A., Profice, P., Ferrara, L., Insola, A., Mazzone, P., Tonali, P., Rothwell, J.C., 1998. Effects of voluntary contraction on descending volleys evoked by transcranial stimulation in conscious humans. Journal of Physiology 508, 625-633.

Furby, A., Bourriez, J.L., Jacquesson, J.M., Mouniervehier, F., Guieu, J.D., 1992. Motor evoked-potentials to magnetic stimulation: Technical considerations and normative data from 50 subjects. Journal of Neurology 239, 152-156.

Gelfert, C.C., Alpers, I., Dallmeyer, M., Decker, M., Huting, A., Lesch, S., Baumgartner, W., Staufenbiel, R., 2007. Factors affecting the success rate of treatment of recumbent dairy cows suffering from hypocalcaemia. Journal of Veterinary Medicine. 54, 191-198.

Grillon, C., Baas, J., 2003. A review of the modulation of the startle reflex by affective states and its application in psychiatry. Clinical Neurophysiology 114, 1557-1579.

Healy, A.M., Doherty, M.L., Monaghan, M.L., McAllister, H., 1997. Cervico-thoracic vertebral osteomyelitis in 14 calves. Veterinary Journal 154, 227-232.

Hess, C.W., Ludin, H.P., 1988. Transcranial brain-stimulation by magnetic pulses - methodological and physiological considerations. Eeg-Emg-Zeitschrift fur Elektroenzephalographie Elektromyographie Und Verwandte Gebiete 19, 209-215.

Matamala, J.M., Nunez, C., Lera, L., Verdugo, R.J., Sanchez, H., Albala, C., Castillo, J.L., 2013. Motor evoked potentials by transcranial magnetic stimulation in healthy elderly people. Somatosensory and Motor Research 30, 201-205.

Matsumoto, H., Hanajima, R., Terao, Y., Ugawa, Y., 2013. Magnetic-motor-root stimulation: Review. Clinical Neurophysiology 124, 1055-1067.

Mayhew, I.G., Washbourne, J.R., 1996. Magnetic motor evoked potentials in ponies. Journal of Veterinary Internal Medicine 10, 326-329.

Mills, K.R., Murray, N.M., Hess, C.W., 1987. Magnetic and electrical transcranial brain stimulation: Physiological mechanisms and clinical applications. Neurosurgery 20, 164-168.

Nardone, R., Holler, Y., Thomschewski, A., Holler, P., Bergmann, J., Golaszewski, S., Brigo, F., Trinka, E., 2014. Central motor conduction studies in patients with spinal cord disorders: A review. Spinal Cord 52, 420-427.

Nollet, H., Deprez, P., Van Ham, L., Verschooten, F., Vanderstraeten, G., 2002. The use of magnetic motor evoked potentials in horses with cervical spinal cord disease. Equine Veterinary Journal 34, 156-163.

Nollet, H., Van Ham, L., Dewulf, J., Vanderstraeten, G., Deprez, P., 2003a. Standardization of transcranial magnetic stimulation in the horse. Veterinary Journal 166, 244-250.

Nollet, H., Van Ham, L., Verschooten, F., Vanderstraeten, G., Deprez, P., 2003b. Use of magnetic motor-evoked potentials in horses with bilateral hind limb ataxia. American Journal of Veterinary Research 64, 1382-1386.

Nollet, H., Deprez, R., Van Ham, L., Dewulf, J., Decleir, A., Vanderstraeten, G., 2004. Transcranial magnetic stimulation: Normal values of magnetic motor evoked potentials in 84 normal horses and influence of height, weight, age and sex. Equine Veterinary Journal 36, 51-57.

Nollet, H., Vanschandevijl, K., Van Ham, L., Vanderstraeten, G., Deprez, P., 2005. Role of transcranial magnetic stimulation in differentiating motor nervous tract disorders from other causes of recumbency in four horses and one donkey. Veterinary Record 157, 656-658.

Parent, J., 2010. Clinical approach and lesion localization in patients with spinal diseases. Veterinary Clinics of North America: Small Animal Practice 40, 733-753.

Preece, A.W., Wimalaratna, H.S., Green, J.L., Churchill, E., Morgan, H.M., 1994. Non-invasive quantitative EMG. Electromyography and Clinical Neurophysiology 34, 81-86.

Preston, D.C., Shapiro, B.E., 2002. Needle electromyography. Fundamentals, normal and abnormal patterns. Neurologic Clinics 20, 361-396.

Rossi, S., Hallett, M., Rossini, P. M., Pascual-Leone, A., 2009. Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. Clinical neurophysiology 120, 2008-2039

Rossini, P.M., Burke, D., Chen, R., Cohen, L.G., Daskalakis, Z., Di Iorio, R., Di Lazzaro, V., Ferreri, F., Fitzgerald, P.B., George, M.S., et al., 2015. Non-invasive electrical and magnetic stimulation of the brain, spinal cord, roots and peripheral nerves: Basic principles and procedures for routine clinical and research application. An updated report from an I.F.C.N. Committee. Clinical Neurophysiology 126, 1071-1107.

Schenk, H.C., Haastert-Talini, K., Jungnickel, J., Grothe, C., Meyer, H., Rehage, J., Fehr, M., Bokemeyer, J., Rohn, C., Tipold, A., 2014. Morphometric parameters of peripheral nerves in calves correlated with conduction velocity. Journal of Veterinary Internal Medicine 28, 646-655.

Sherman, D.M., 1987. Localized diseases of the bovine brain and spinal cord. Veterinary Clinics of North America: Food Animal Practice 3, 179-191.

Ugawa, Y., Uesaka, Y., Terao, Y., Hanajima, R., Kanazawa, I., 1997. Magnetic stimulation of the descending and ascending tracts at the foramen magnum level. Electroencephalography and Clinical Neurophysiology 105, 128-131.

van Dijk, J.G., Tjon-a-Tsien, A., van der Kamp, W., 1995. CMAP variability as a function of electrode site and size. Muscle Nerve 18, 68-73.

Van Ham, L.M., Nijs, J., Mattheeuws, D.R., Vanderstraeten, G.G., 1996. Sufentanil and nitrous oxide anaesthesia for the recording of transcranial magnetic motor evoked potentials in dogs. Veterinary Record 138, 642-645.

Van Ham, L.M.L., Vanderstraeten, G.G.W., Mattheeuws, D.R.G., Nijs, J., 1994. Transcranial magnetic motor evoked-potentials in sedated dogs. Progress in Veterinary Neurology 5, 147-154.

CHAPTER 4

MAGNETIC MOTOR EVOKED POTENTIALS OF CERVICAL MUSCLES IN HORSES

MAGNETIC MOTOR EVOKED POTENTIALS OF CERVICAL MUSCLES IN HORSES

J. Rijckaert[1], B. Pardon[1], L. Van Ham[2], P. Joosten[3], G. van Loon[1], P. Deprez[1]

[1] Department of Large Animal Internal Medicine, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, Merelbeke 9820, Belgium

[2] Small Animal Department, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, Merelbeke 9820, Belgium

[3] Department of Obstetrics, Reproduction and Herd Health, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, Merelbeke 9820, Belgium

Adapted from: Rijckaert, J., Pardon, B., Van Ham, L., Joosten, P., van Loon, G., Deprez, P., 2018. Magnetic motor evoked potentials of cervical muscles in horses. BMC veterinary research 14, 290.

ABSTRACT

Background - When surgical treatment of cervical vertebral malformation is considered, precise localization of compression sites is essential, but remains challenging. Magnetic motor evoked potentials (MMEPs) from paravertebral muscles are useful in localizing spinal cord lesions, but no information about cervical muscle MMEPs in horses is available yet. Therefore, the aim of this study was to determine the possibility, normal values, inter- and intra-observer agreement and factors that have an effect on cervical MMEPs in healthy horses.

Methods - Transcranial magnetic stimulation was performed on 50 normal horses and 4 (2 left, 2 right) muscle responses were recorded at the middle of each cervical vertebra (C1-C7) and additionally just caudal to C7 to evaluate cervical nerves (Cn) Cn1 to Cn8. Latency time and amplitude of the recorded MMEPs were defined by both an experienced and an unexperienced operator.

Results - Latency increased gradually from 14.2±1.38 ms for Cn3 to 17.7±1.36 ms for Cn8, was significantly influenced by cervical nerve (P<0.01), gender (P=0.02) and height (P=0.03) and had a good intra-observer agreement. The smallest mean amplitude (4.35±2.37 mV) was found at Cn2, the largest (5.99±2.53 mV) at Cn3. Amplitude was only significantly influenced by cervical nerve (P<0.01) and had a low intra-observer agreement. No significant effect of observer on latency (P=0.88) or amplitude (P=0.99) measurements was found.

Conclusion - MMEPs of cervical muscles in normal horses are easy to collect and to evaluate with limited intra- and inter-observer variation concerning latency time and should be investigated in future studies in ataxic horses to evaluate its clinical value.

Keywords:

EMG, neurologic test, spinal ataxia, surgery, transcranial magnetic stimulation

INTRODUCTION

In human patients with compressive myelopathy, spinal cord vascular disorders, myelitis or spinal cord injury, medical diagnostic imaging provides detailed information, but often shows compression in asymptomatic lesions (Barker et al., 1985; Boden et al., 1990; Nardone et al., 2014; Tani et al., 1999). Also in horses, many studies highlight the controversy, difficulties and limitations of cervical radiography, myelography, computed tomography (CT), magnetic resonance imaging (MRI) and myeloscopy to diagnose spinal cord disease. Sensitivities (47-50%) and specificities (70-78%) of cervical radiographs and sagittal ratio calculations are too low for adequate diagnosis of spinal cord compression (Levine et al., 2007; Levine et al., 2010) and variation between observers is high (Hughes et al., 2014a). Myelography also has a low sensitivity (43-85%) and additionally requires general anesthesia and intrathecal contrast injection (Levine et al., 2010; van Biervliet et al., 2004). Most CT and MRI scanners can only image the cranial cervical spinal cord because of the limited diameter of the CT and MRI gantry. This is an important limitation since 37-54% of CVM lesions occur in the caudal (C5-C7) cervical vertebral column (Levine et al., 2010; van Biervliet et al., 2004). Furthermore, no flexion or extension of the neck is possible in CT or MRI scanning (Janes et al., 2014; Mitchell et al., 2012b; Yamada et al., 2016). Cervical vertebral canal endoscopy is not routinely performed as there is a high risk of complications associated with entering the spinal canal or due to neck movement during the procedure. In addition, the visual assessment of subarachnoid space narrowing may not be reliable in cases with mild to moderate stenosis (Prange et al., 2012).

On the other hand, non-infectious spinal ataxia and paresis is an important issue in horses. Major causes include trauma, neoplasia or equine degenerative myeloencephalopathy, but in European horses, spinal ataxia is most commonly caused by cervical vertebral malformation (CVM). CVM is a stenosis of the cervical vertebral canal with static or dynamic compression of the spinal cord (Nout and Reed, 2003) commonly seen in young, rapidly growing thoroughbreds and in warmblood horses (Levine et al., 2007; Levine et al., 2008; Levine et al., 2010). Thoroughbreds (6 months–2 years) are prone to a dynamic compressive form (type 1), while the static form (type 2) typically affects older warmblood horses in the caudal cervical region and is caused by osteoarthritic enlargement of the cervical articular processes (Levine et al., 2007; Mayhew et al., 1993).

Hoffman and Clark (2013) described that some thoroughbreds (about 30%) were able to race at least once after CVM diagnosis and non-surgical management, but if bony malformations or soft tissue proliferations exist, neurological deficits remain present (Nout and Reed, 2003) and many horses are euthanized (Hoffman and Clark, 2013; Levine et al., 2010). Surgical treatment can improve prognosis: Moore et al. (1993) and Walmsley (2005) described that 45-60% of the patients who underwent vertebral body fusion returned to use, but the clinical response to surgery depended strongly on the ability to identify all compressed sites (Moore et al., 1993; Walmsley, 2005). Although this precise localization is essential, it still remains challenging because of the limitations of medical imaging. Therefore, it is essential to correlate results of medical imaging with neurophysiological examinations (Chan et al., 1998; Nardone et al., 2014).

A highly accurate diagnostic test with a very high sensitivity in human patients is transcranial magnetic stimulation which evokes synchronized descending volleys in corticospinal pathways: magnetic motor evoked potentials (MMEPs) (Di Lazzaro et al., 1999). MMEPs with prolonged latencies indicate pathological slowing of the conduction through the corticospinal tract in a non-invasive way (Dvorak et al., 1990). The test might be contra-indicated in epileptic patients and in patients with pacemakers or implanted metal structures in the brain but the incidence of side effects is low. By using specific muscles to record MMEPs, such as paravertebral muscles, and by determination of central and peripheral motor conduction time, exact localization of lesions and distinction between central and peripheral neural pathology is possible in human patients (Funaba et al., 2015b; Nardone et al., 2014). In ataxic or paralytic horses, lesions have already grossly been localized using MMEP conductions into thoracic and pelvic limbs (Nollet et al., 2002; Nollet et al., 2003c; Nollet et al., 2005). Best results are obtained after sedation with detomidine (1mg/100 kg) and buprenorphine (0.24 mg/100 kg) and stimulation with a round 70 mm diameter coil placed high on the frontal region of the horse, with a maximal output of the stimulator (maximal magnetic field of 4 Tesla) (Nollet et al., 2004; Nollet et al., 2003a; Nollet et al., 2003b). Coil current had no significant effect on latency values and no adverse effects have been reported (Nollet et al., 2003a). Abnormal thoracic and pelvic limb muscle MMEP latency and amplitude values suggest cervical spinal cord disease (Nollet et al., 2002) while pelvic muscle abnormalities alone, occur with thoracic or thoracolumbar pathology. However, no differentiation between

etiology or central or peripheral lesions can be made (Nollet et al., 2003c). Of course, it would be interesting to localize the lesion more precisely in horses, just like in human medicine, using cervical paravertebral muscles MMEPs. These muscles are situated close to the vertebral column making the peripheral component of the nervous system small. This means that, if spinal root compression is absent, these cervical muscle MMEPs will approximate the central motor conduction time, making distinction between central and peripheral pathology possible. However, this paravertebral muscles examination is currently unexplored in horses. Therefore, the aim of this study was to investigate whether paravertebral muscle MMEP recording is possible in horses and, if so, to determine normal values, inter- and intra-observer agreement and factors that might have an influence on MMEP recordings of paravertebral cervical muscles in healthy horses.

MATERIAL AND METHODS

ANIMALS

To determine reference values for MMEPS in cervical muscles of horses, sample size was set at 50 as recommended (Wellek et al., 2014). Healthy horses (36 mares, 12 geldings and 2 stallions; 35 warmbloods, 11 trotters, 1 pony, 1 Friesian, 1 Arabian and 1 Andalusian) were conveniently selected. Thirty horses were owned by the faculty of veterinary medicine of Ghent University as laboratory animals, the other 20 horses were recipient mares for embryo transfers loaned by Keros plc. All horses returned to their owners 1 day after the test. The median age of the horses was 11.5 (range 3 to 22, 15 horses between 3 and 7, 14 between 8 and 12, 17 between 13 and 17 and 4 between 18 and 22) years, the median height 160 (range 142 to 175) cm and median weight 553 (range 388 to 705) kg. All horses had a body condition score between 3/9 and 6/9. There were no significant age or height differences between males and females. The mean weight of male horses was significantly (P<0.01) higher than the mean weight of the female horses (557 ± 79 versus 546 ± 76 kg). Rectal temperature was normal ranging from 37.1 to 37.9°C. All horses were examined clinically and neurologically by a veterinarian with 3 years of experience in neurological examinations. The neurological evaluation form of Mayhew (2008) was used as guideline. During the examination, special

attention was paid to a normal mobility and the absence of any swelling of the neck. Only clinically and neurologically normal horses were included in this study.

MAGNETIC STIMULATION AND MMEP RECORDING

Each horse was sedated intravenously with a combination of detomidine (Domidine, Eurovet Animal Health, 12µg/kg bwt) and butorphanol (Dolorex, MSD Animal Health, 12µg/kg bwt). After 5 minutes, the level of sedation of the horse was subjectively evaluated. Horses that were still alert and reactive to a hand clap, received an additional 6µg/kg detomidine and 6µg/kg butorphanol. For the magnetic stimulation and MMEP recording, the horses were all placed in the same examination room with an environmental temperature ranging from 18-24°C. A magnetic stimulator (Magstim 200, The Magstim Company) and a round 70 mm coil were used to generate a maximal magnetic field of 4 Tesla at the coil surface. The coil was centered over the dorsal part of the frontal bone as described by Nollet et al. (2003a) and maximal stimulus intensity (100%) was applied. The muscle responses were recorded by a standard electromyograph (EMG; Medelec Sapphire, Medelec) through needle electrodes at the level of the middle of each vertebra (C1-C7) and additionally just caudal to the 7th vertebra (C7) to evaluate cervical nerves (Cn) Cn1 to Cn8. The active needle electrode (disposable monopolar needle electrode, 37mm, 26G, TECA Corporation) was placed as deep as possible (full length or until contact with the vertebral bone, when it was pulled back 2 mm) to reach the paravertebral *intertransversarii cervicii* muscles of C3-C7. The accessibility of these paravertebral muscles was confirmed with ultrasound based on the study of Berg et al. (2003). At the level of the atlas and axis, the needle was placed as deep as possible in the *obliquus capitis cranialis* and *caudalis* muscle, as there are no paravertebral *intertransversarii* muscles at this level. The reference electrode (disposable monopolar needle electrode, 25mm, 26G, TECA Corporation) was placed subcutaneously at the level of the active electrode. The ground electrode was always attached at the level of the tuber olecranon. Localization of the measurement points was done by palpating the transversal processes of the cervical vertebra and visually determining the middle between 2 sequential processes. At each vertebra, two responses were recorded at the left side and two at the right side of the horse.

Latency and amplitude were defined for each response. Latency (ms) was defined as the time between the stimulation and the onset of contraction, indicated by the first deflection from the baseline. Amplitude (mV) was defined as the difference between the two largest peaks of opposite polarity. All stimulations were carried out by observer 1. On all recorded MMEPS, marker positioning for latency and amplitude were done independently by the same two operators. Observer 1 had 3 years of experience in neurological examinations and recording of MMEPs, observer 2 had no experience.

STATISTICS

Data were entered on a spreadsheet (Excel, Microsoft Corporation) and transferred to SPSS 2.4 (IBM SPSS Statistics for Windows) for descriptive and statistical analysis. The continuous outcome variables latency and amplitude were checked for a normal distribution (Kolmogorov Smirnov test and inspection of Q-Q plots and histograms). To determine the 90% reference intervals (upper and lower bound) for latency and amplitude, an Excel add-in was used (Reference Value Advisor; Geffré et al., 2011) and the shortest latency time and the highest amplitude of the four stimulations per location per horse were used. The programme calculates untransformed, box transformed and non-parametric reference intervals and indicates which intervals comply best with the data set.

To determine factors which are significantly associated with latency and amplitude, linear mixed models were used with horse and cervical nerve as random effect to account for clustering of measurements within a horse. Predictors added to the model were cervical nerve (1-8), side (left or right), sedation dose, breed and the physiological parameters gender, age (years), body weight (kg) and withers height (cm). First, all parameters were tested univariably. Then, all parameters with $P<0.20$ were retained for the final multivariable model, which was built stepwise backwards gradually, excluding non-significant factors. When predictor variables were highly correlated (Pearson correlation > 0.60), only the most significant variable was added to the model. Biologically relevant interactions of significant main effects were tested. For all significant categorical variables, pairwise comparisons were made using post-hoc tests with a Bonferroni correction to adjust for multiple comparisons.

Inter- and intra-observer agreements were determined for each location (four measurements per vertebra), using linear mixed model procedures and coefficients of variation (CV (%)=(SD/mean) x 100), respectively. CVs were calculated on horse level by taking the mean value of the CV per horse and on study population level by calculating the CV of the minimal latency and maximal amplitude values of 4 measurements per horse.

RESULTS

A total of 1600 stimulations were performed and all delivered measurable MMEPs. Latency and amplitude recordings for each cervical nerve are displayed in Figures 1 and 2 and descriptive statistics and calculated 90% reference intervals are presented in Table 1. Factors univariably associated with latency were cervical nerve (P<0.001), gender (P=0.07), age (P=0.05), height (P<0.001), weight (P<0.001) and sedation dose (P<0.001). In the final multivariable model for latency, only the factors cervical nerve (P<0.01), gender (P=0.02) and height (P=0.03) remained significantly associated. Interactions between the different significant main effects were tested, but only the interaction between cervical nerve and gender was significant (P<0.01) (Figure 3). This interaction signifies a different effect of the cervical nerve location on latency time for males and females. In male horses Cn2, Cn5, Cn6, Cn7 and Cn8 had significantly (P<0.01) longer latency times with mean differences of respectively 0.7, 1.1, 1.0, 1.1 and 1.5 ms compared to females. Irrespective of gender, latency increases gradually from 14.2 ms in Cn3 to 17.7 ms in Cn8 with significant differences between the different locations. Cn1 and Cn2 were not significantly different, but Cn2 had significantly longer latency values than Cn3 (Figure 1).

Data for amplitude needed to be log transformed to obtain a normal distribution. Factors univariably associated with amplitude were cervical nerve (P<0.01) and sedation dose (P=0.15). In the final multivariable model only cervical nerve was significant (P<0.01). Amplitude was smallest (4.35 mV) in Cn2 and largest (5.99 mV) in Cn3. Cn2 had significantly different amplitude values compared to Cn3, Cn6, Cn7 and Cn8. Mean maximal amplitude for Cn3 was also significantly different from Cn1 and Cn4, and for Cn1 it was significantly different from Cn8.

Mixed models showed no significant effect of observer on latency (*P*=0.88) or amplitude (*P*=0.99). CV, on horse and on study population level, of latency and amplitude measurements for both observers are represented in Table 2.

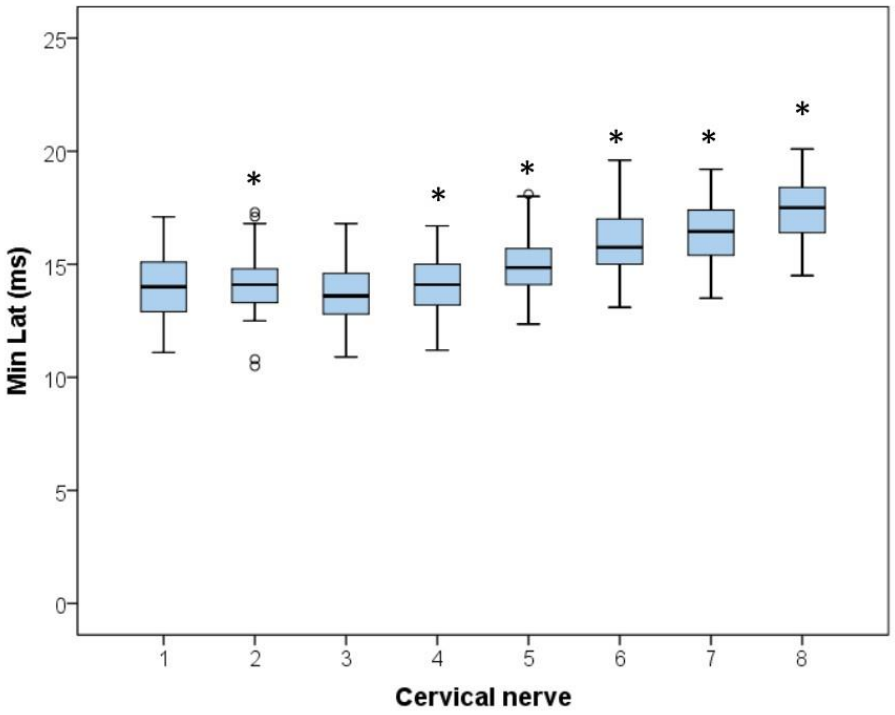


Figure 1. Boxplots for all latency values per cervical nerve (8 times *n*=200). Significant differences (*P*<0.01) from group 3, the group with the lowest latency values, are indicated by *, outliers are indicated by a circle.

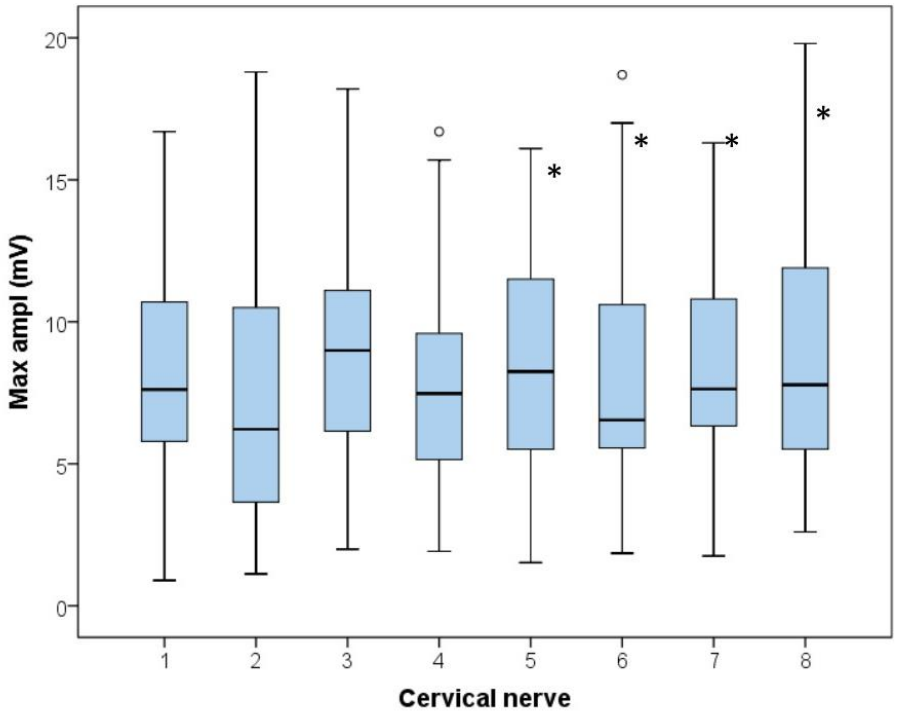


Figure 2. Boxplots for all amplitude values per cervical nerve (8 times *n*=200). Analogous to latency, significant differences (*P*<0.01) from group 3 are indicated by *, outliers are indicated by a circle.

Table 1.

Mean observed values, standard deviations (SD), minimum (Min) and maximum (Max) values, and calculated reference intervals (RI) for latency (shortest latency of 4 observations per horse) and amplitude (maximum amplitude of 4 observations per horse) values for each cervical nerve in 50 healthy horses.

	Cervical nerve	*n*	Mean	SD	Min	Max	90% RI
Latency (ms)	1	50	14.0	1.4	11.1	17.1	11.2-16.8
	2	50	14.1	1.4	10.5	17.3	10.6-17.2
	3	50	13.7	1.4	10.9	16.8	11.0-16.6
	4	50	14.1	1.2	11.2	16.7	11.4-16.6
	5	50	15.0	1.3	12.4	18.1	12.6-18.1
	6	50	15.9	1.5	13.1	19.6	13.2-19.4
	7	50	16.5	1.4	13.5	19.2	13.6-19.2
	8	50	17.4	1.3	14.5	20.1	14.6-20.1
Amplitude (mV)	1	50	8.2	3.7	0.9	16.7	1.2-16.2
	2	50	7.1	4.3	1.1	18.8	1.1-18.3
	3	50	9.3	4.0	2.0	18.2	2.2-18.0
	4	50	7.8	3.4	1.9	16.7	2.0-16.4
	5	50	8.4	3.9	1.5	16.1	1.6-15.9
	6	50	8.0	3.9	1.9	18.7	2.1-18.2
	7	50	8.3	2.9	1.8	16.3	2.1-15.4
	8	50	8.8	4.1	2.6	19.8	2.7-19.8

n, number of magnetic motor evoked potentials recorded

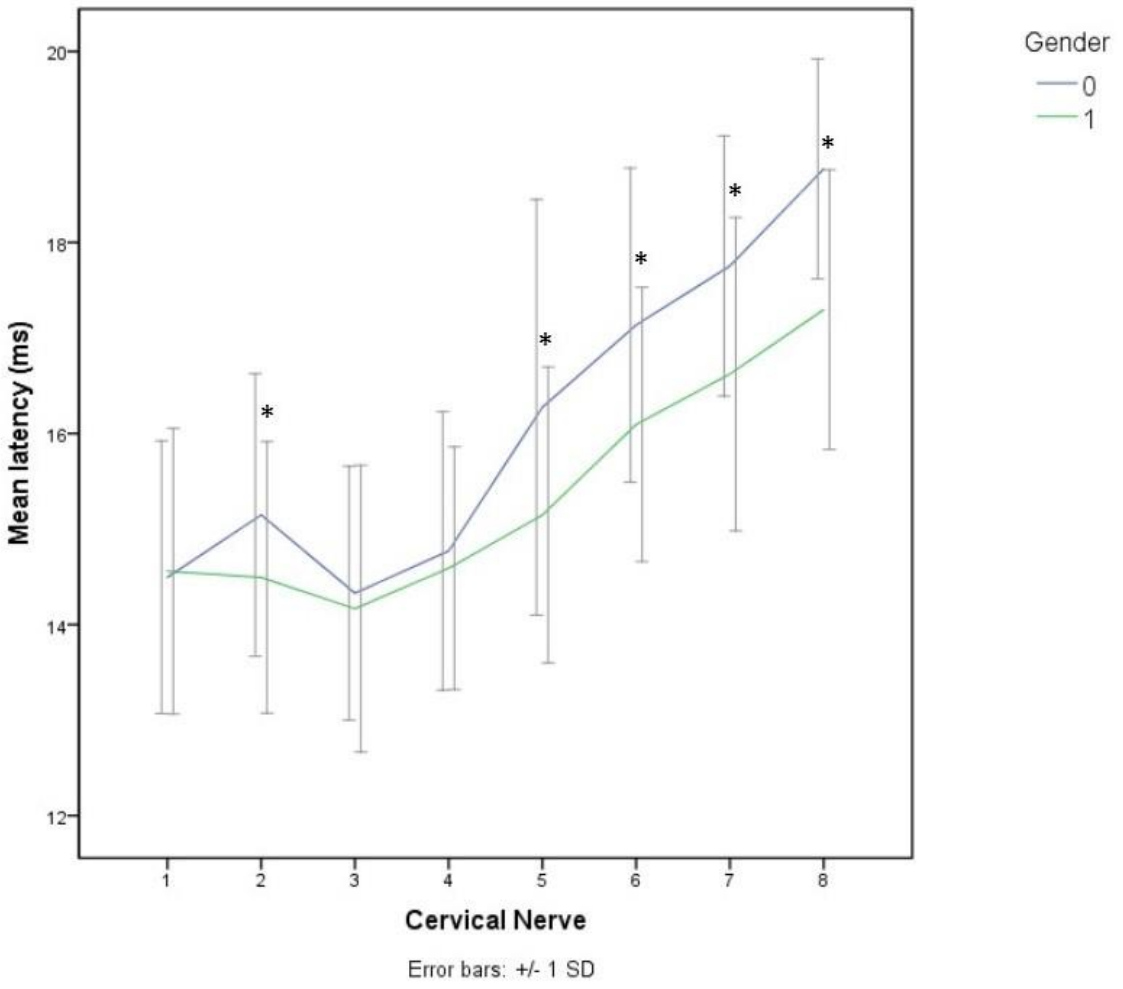


Figure 3. Interaction between gender (0=male and 1=female) and cervical nerve on latency. Significant differences (*P*<0.05*)* are demonstrated by *.

Table 2.

Coefficients of variation (CV (%)=SD/mean*100) per cervical nerve (Cn) of latency and amplitude on horse (mean of CVs calculated per horse) and study population (CV of minimal latency and maximum amplitude values per horse) level for observer 1 and 2.

	Level	Observer	Cn1	Cn2	Cn3	Cn4	Cn5	Cn6	Cn7	Cn8	Mean
Latency	Horse	1	3.10	3.00	2.70	3.19	3.76	2.48	3.16	2.24	2.79
		2	4.53	4.10	4.05	4.60	4.09	3.00	3.15	2.16	3.71
	Study population	1	9.71	9.87	10.14	8.81	8.76	9.31	8.36	7.67	9.08
		2	14.50	13.77	14.50	12.99	15.20	10.89	9.75	8.41	12.50
Amplitude	Horse	1	48.54	43.61	39.02	40.51	44.01	33.97	38.95	38.44	36.72
		2	49.61	44.57	39.95	40.39	43.18	36.96	39.31	38.44	41.55
	Study population	1	44.42	59.76	43.13	43.00	46.13	48.52	35.04	46.87	45.86
		2	57.26	60.32	43.23	44.22	46.83	46.02	36.78	61.21	49.48

DISCUSSION

Transcranial magnetic stimulation with registration of MMEPs in cervical muscles is possible and easy to perform in horses, as all stimulations resulted in muscle responses from which latency and amplitude could be measured. All measurements of latency and amplitude were carried out by an experienced and an unexperienced observer. For latency, the variation per cervical nerve within a horse was very small for each observer, indicating good intra-observer repeatability. General CVs in this population of horses were larger but still small enough to state that latency was sufficiently repeatable (Ashcroft et al., 1988; Mavrides et al., 2001). These findings correspond to the small variations in repeated recordings found in previous equine MMEP studies (Hess et al., 1986; Nollet et al., 2002; Nollet et al., 2004; Rijckaert et al., 2018b). Also inter-observer reproducibility was very good as mixed models did not show a significant observer effect. So, in this study, no significant observer effects could be demonstrated suggesting that latency is a reproducible parameter for cervical MMEPs, even for clinicians without experience.

Latency values were influenced by height, cervical nerve and gender with a significant interaction between cervical nerve and gender. The significant effect of height was already found in horses (Nollet et al., 2004) and humans (Ellaway et al., 1998). The increasing neural length along the spinal cord, results in a progressive increase in latency (Ertekin et al., 1998). This was confirmed in our study although no measurements of exact neural or neck length were performed. In general, the increase in latency was relatively small (3.3 ms) and started only from Cn3. Mean values for Cn1 and Cn2 were significantly larger than for Cn3. Obviously the anatomy of the atlas and axis region is totally different compared to the more caudal parts of the neck. So, at the level of C1 and C2, a different muscle, which might also be different in composition and innervation, was tested than more caudally along the neck. The influence of gender was not found previously in horses (Nollet et al., 2004) nor in calves (Sylvestre et al., 1993), but has been described in human subjects (Chu, 1989; Tobimatsu et al., 1998), though most studies showed no gender effect once corrected for height (Rossini et al., 2015). In the present study, no differences in height were found between males and females, but a significant weight difference was present. The most likely hypothesis is that male horses have a higher level of muscularity (hence the higher body weight) which might result in a longer distance from stimulation to recording site and hence to longer latencies. The longer latency

times in male horses became even clearer in the caudal, stronger muscled, parts of the neck supporting this hypothesis.

The reference intervals for latency were calculated and can be used in clinical situations in future. The 5-6 ms variation in latency values in normal horses is relatively large and probably caused by the large range in horse height in this study population and a sample size at the lower half of the recommended number for reference interval determinations (Wellek et al., 2014). In addition, subjective localization of the measuring points, based on palpation of the transverse processes and subjectively determining the middle of each vertebra, differences in local temperature or subclinical lesions may have contributed to some variation. However, this variation is probably of limited clinically importance in horses with severe cervical spinal cord disease as in such horses latency times commonly double [when recorded from caudal to the lesion] compared to normal values (Nollet et al., 2002). Only in subtle or subclinical cases, the variation might result in false negative results. This needs to be determined by validation studies in future, by including acute and chronic and mild and severe clinical case material.

With regard to amplitude, intra-observer repeatability was poor for both horse and general level. The large CVs for amplitude are a logical consequence of the large standard deviations compared to the mean values. Large CVs for amplitude were also found in normal human patients (Ellaway et al., 1998; Hess et al., 1986), dogs (Sylvestre et al., 1993; Van Ham et al., 1996a; Van Ham et al., 1994) and previous MMEP studies in large animals (Nollet et al., 2002; Nollet et al., 2004; Nollet et al., 2003a; Nollet et al., 2003b; Rijckaert et al., 2018b; Rijckaert et al., 2016). This large variation is possibly due to physical changes in stimulation (inter-trial variations: output of the stimulator, position of the coil, position of the needle electrodes) or neurophysiological differences in the patient (inter- and intra-individual variations: level of relaxation or voluntary contraction, excitability variations)(Ellaway et al., 1998; Nollet et al., 2004; Wellek et al., 2014). However, these factors are difficult or impossible to control in a clinical setting. As a consequence, the calculated reference intervals are wide. In contrast, inter-observer reproducibility of amplitude measurements was good as mixed models showed no significant influence of observer. Thus, results for amplitude measurements are similar for experienced and non-experienced clinicians, but the clinical value of this parameter remains limited because of its high variation.

CONCLUSION

Registration and measurement of latency and amplitude values from MMEPs of cervical muscles in normal horses is easy, with limited intra- and inter-observer variation. In future, the available reference values for healthy horses can be compared with values in horses with suspected cervical spinal cord disease to evaluate whether precise localization of lesions is possible.

REFERENCES

Ashcroft, T., Simpson, J.M., Timbrell, V., 1988. Simple method of estimating severity of pulmonary fibrosis on a numerical scale. Journal of Clinical Pathology41, 467-470.

Barker, A.T., Jalinous, R., Freeston, I.L., 1985. Non-invasive magnetic stimulation of human motor cortex. Lancet 1, 1106-1107.

Berg, L.C., Nielsen, J.V., Thoefner, M.B., Thomsen, P.D., 2003. Ultrasonography of the equine cervical region: a descriptive study in eight horses. Equine Veterinary Journal 35, 647-655.

Boden, S.D., McCowin, P.R., Davis, D.O., Dina, T.S., Mark, A.S., Wiesel, S., 1990. Abnormal magnetic-resonance scans of the cervical spine in asymptomatic subjects. A prospective investigation. The Journal of bone and joint surgery. American volume 72, 1178-1184.

Chan, K.M., Nasathurai, S., Chavin, J.M., Brown, W.F., 1998. The usefulness of central motor conduction studies in the localization of cord involvement in cervical spondylytic myelopathy. Muscle & nerve 21, 1220-1223.

Chu, N.S., 1989. Motor evoked potentials with magnetic stimulation: correlations with height. Electroencephalography and Clinical Neurophysiology 74, 481-485.

Di Lazzaro, V., Oliviero, A., Profice, P., Ferrara, L., Saturno, E., Pilato, F., Tonali, P., 1999. The diagnostic value of motor evoked potentials. Clinical Neurophysiology110, 1297-1307.

Dvorak, J., Herdmann, J., Theiler, R., 1990. Magnetic transcranial brain stimulation: painless evaluation of central motor pathways. Normal values and clinical application in spinal cord diagnostics: upper extremities. Spine 15, 155-160.

Ellaway, P.H., Davey, N.J., Maskill, D.W., Rawlinson, S.R., Lewis, H.S., Anissimova, N.P., 1998. Variability in the amplitude of skeletal muscle responses to magnetic stimulation of the motor cortex in man. Electroencephalography and Clinical Neurophysiology 109, 104-113.

Ertekin, C., Uludag, B., On, A., Yetimalar, Y., Ertas, M., Colakoglu, Z., Arac, N., 1998. Motor-evoked potentials from various levels of paravertebral muscles in normal subjects and in patients with focal lesions of the spinal cord. Spine 23, 1016-1022.

Funaba, M., Kanchiku, T., Imajo, Y., Suzuki, H., Yoshida, Y., Nishida, N., Taguchi, T., 2015. Transcranial magnetic stimulation in the diagnosis of cervical compressive myelopathy: comparison with spinal cord evoked potentials. Spine 40, E161-167.

Hess, C.W., Mills, K.R., Murray, N.M., 1986. Magnetic stimulation of the human brain: facilitation of motor responses by voluntary contraction of ipsilateral and contralateral muscles with additional observations on an amputee. Neuroscience Letters 71, 235-240.

Hoffman, C.J., Clark, C.K., 2013. Prognosis for racing with conservative management of cervical vertebral malformation in thoroughbreds: 103 cases (2002-2010). Journal of Veterinary Internal Medicine 27, 317-323.

Hughes, K.J., Laidlaw, E.H., Reed, S.M., Keen, J., Abbott, J.B., Trevail, T., Hammond, G., Parkin, T.D., Love, S., 2014. Repeatability and intra- and inter-observer agreement of cervical vertebral sagittal diameter ratios in horses with neurological disease. Journal of Veterinary Internal Medicine 28, 1860-1870.

Janes, J.G., Garrett, K.S., McQuerry, K.J., Pease, A.P., Williams, N.M., Reed, S.M., MacLeod, J.N., 2014. Comparison of magnetic resonance imaging with standing cervical radiographs for evaluation of vertebral canal stenosis in equine cervical stenotic myelopathy. Equine Veterinary Journal 46, 681-686.

Levine, J.M., Adam, E., MacKay, R.J., Walker, M.A., Frederick, J.D., Cohen, N.D., 2007. Confirmed and presumptive cervical vertebral compressive myelopathy in older horses: A retrospective study (1992-2004). Journal of Veterinary Internal Medicine 21, 812-819.

Levine, J.M., Ngheim, P.P., Levine, G.J., Cohen, N.D., 2008. Associations of sex, breed, and age with cervical vertebral compressive myelopathy in horses: 811 cases (1974-2007). Journal of the American Veterinary Medical Association 233, 1453-1458.

Levine, J.M., Scrivani, P.V., Divers, T.J., Furr, M., Mayhew, I.J., Reed, S., Levine, G.J., Foreman, J.H., Boudreau, C., Credille, B.C., Tennent-Brown, B., Cohen, N.D., 2010. Multicenter case-control study of signalment, diagnostic features, and outcome associated with cervical vertebral malformation-malarticulation in horses. Journal of the American Veterinary Medical Association 237, 812-822.

Mavrides, E., Holden, D., Bland, J.M., Tekay, A., Thilaganathan, B., 2001. Intraobserver and interobserver variability of transabdominal Doppler velocimetry measurements of the fetal ductus venosus between 10 and 14 weeks of gestation. Ultrasound in obstetrics & gynecology 17, 306-310.

Mayhew, I.G., 2008. Large Animal Neurology - neurological evaluation form, 2nd ed. Wiley-Blackwell, Chichester.

Mayhew, I.G., Donawick, W.J., Green, S.L., Galligan, D.T., Stanley, E.K., Osborne, J., 1993. Diagnosis and prediction of cervical vertebral malformation in thoroughbred foals based on semi-quantitative radiographic indicators. Equine Veterinary Journal 25, 435-440.

Mitchell, C.W., Nykamp, S.G., Foster, R., Cruz, R., Montieth, G., 2012. The Use of Magnetic Resonance Imaging in Evaluating Horses with Spinal Ataxia. Veterinary Radiology and Ultrasound 53, 613-620.

Moore, B.R., Reed, S.M., Robertson, J.T., 1993. Surgical treatment of cervical stenotic myelopathy in horses: 73 cases (1983-1992). Journal of the American Veterinary Medical Association 203, 108-112.

Nardone, R., Holler, Y., Thomschewski, A., Holler, P., Bergmann, J., Golaszewski, S., Brigo, F., Trinka, E., 2014. Central motor conduction studies in patients with spinal cord disorders: a review. Spinal cord 52, 420-427.

Nollet, H., Deprez, P., Van Ham, L., Verschooten, F., Vanderstraeten, G., 2002. The use of magnetic motor evoked potentials in horses with cervical spinal cord disease. Equine Veterinary Journal 34, 156-163.

Nollet, H., Deprez, R., Van Ham, L., Dewulf, J., Decleir, A., Vanderstraeten, G., 2004. Transcranial magnetic stimulation: normal values of magnetic motor evoked potentials in 84 normal horses and influence of height, weight, age and sex. Equine Veterinary Journal 36, 51-57.

Nollet, H., Van Ham, L., Dewulf, J., Vanderstraeten, G., Deprez, P., 2003a. Standardization of transcranial magnetic stimulation in the horse. Veterinary Journal 166, 244-250.

Nollet, H., Van Ham, L., Gasthuys, F., Dewulf, J., Vanderstraeten, G., Deprez, P., 2003b. Influence of detomidine and buprenorphine on motor-evoked potentials in horses. Veterinary Record 152, 534-537.

Nollet, H., Van Ham, L., Verschooten, F., Vanderstraeten, G., Deprez, P., 2003c. Use of magnetic motor-evoked potentials in horses with bilateral hind limb ataxia. American Journal of Veterinary Research 64, 1382-1386.

Nollet, H., Vanschandevijl, K., Van Ham, L., Vanderstraeten, G., Deprez, P., 2005. Role of transcranial magnetic stimulation in differentiating motor nervous tract disorders from other causes of recumbency in four horses and one donkey. Veterinary Record 157, 656-658.

Nout, Y.S., Reed, S.M., 2003. Cervical vertebral stenotic myelopathy. Equine Veterinary Education 15, 212-223.

Prange, T., Carr, E.A., Stick, J.A., Garcia-Pereira, F.L., Patterson, J.S., Derksen, F.J., 2012. Cervical vertebral canal endoscopy in a horse with cervical vertebral stenotic myelopathy. Equine Veterinary Journal 44, 116-119.

Rijckaert, J., Pardon, B., Verryken, K., Van Ham, L., van Loon, G., Deprez, P., 2016. Motor evoked potentials in standing and recumbent calves induced by magnetic stimulation at the foramen magnum. Veterinary Journal 216, 178-182

Rijckaert J, Pardon B, Ham LV, van Loon G, Deprez P. Magnetic Motor Evoked Potential Recording in Horses Using Intramuscular Needle Electrodes and Surface Electrodes. Journal of Equine Veterinary Science. 2018;68:101-7..

Rossini, P.M., Burke, D., Chen, R., Cohen, L.G., Daskalakis, Z., Di Iorio, R., Di Lazzaro, V., Ferreri, F., Fitzgerald, P.B., George, M.S., Hallett, M., Lefaucheur, J.P., Langguth, B., Matsumoto, H., Miniussi, C., Nitsche, M.A., Pascual-Leone, A., Paulus, W., Rossi, S., Rothwell, J.C., Siebner, H.R., Ugawa, Y., Walsh, V., Ziemann, U., 2015. Non-invasive electrical and magnetic stimulation of the brain, spinal cord, roots and peripheral nerves: Basic principles and procedures for routine clinical and research application. An updated report from an I.F.C.N. Committee. Clinical Neurophysiology126, 1071-1107.

Sylvestre, A.M., Cockshutt, J.R., Parent, J.M., Brooke, J.D., Holmberg, D.L., Partlow, G.D., 1993. Magnetic motor evoked potentials for assessing spinal cord integrity in dogs with intervertebral disc disease. Veterinary Surgery 22, 5-10.

Tani, T., Yamamoto, H., Kimura, J., 1999. Cervical spondylotic myelopathy in elderly people: a high incidence of conduction block at C3-4 or C4-5. Journal of Neurology Neurosurgery and Psychiatry 66, 456-464.

Tobimatsu, S., Sun, S.J., Fukui, R., Kato, M., 1998. Effects of sex, height and age on motor evoked potentials with magnetic stimulation. Journal of Neurology 245, 256-261.

van Biervliet, J., Scrivani, P.V., Divers, T.J., Erb, H.N., de Lahunta, A., Nixon, A., 2004. Evaluation of decision criteria for detection of spinal cord compression based on cervical myelography in horses: 38 cases (1981-2001). Equine Veterinary Journal 36, 14-20.

Van Ham, L.M., Nijs, J., Mattheeuws, D.R., Vanderstraeten, G.G., 1996. Sufentanil and nitrous oxide anaesthesia for the recording of transcranial magnetic motor evoked potentials in dogs. Veterinary Record 138, 642-645.

Van Ham, L.M., Vanderstraeten, G.G.W., Mattheeuws, D.R.G., Nijs, J., 1994. Transcranial Magnetic Motor Evoked-Potentials in Sedated Dogs. Progress in veterinary neurology 5, 147-154.

Walmsley, J.R., 2005. Surgical treatment of cervical spinal cord compression in horses: a European experience. Equine Veterinary Education 17, 39-43.

Wellek, S., Lackner, K.J., Jennen-Steinmetz, C., Reinhard, I., Hoffmann, I., Blettner, M., 2014. Determination of reference limits: statistical concepts and tools for sample size calculation. Clinical Chemistry and Laboratory Medicine 52, 1685-1694.

Yamada, K., Sato, F., Hada, T., Horiuchi, N., Ikeda, H., Nishihara, K., Sasaki, N., Kobayashi, Y., Nambo, Y., 2016. Quantitative evaluation of cervical cord compression by computed tomographic myelography in Thoroughbred foals.Journal of Equine Science 27, 143-148.

CHAPTER 5

MAGNETIC MOTOR EVOKED POTENTIAL RECORDING IN HORSES USING INTRAMUSCULAR NEEDLE ELECTRODES AND SURFACE ELECTRODES

MAGNETIC MOTOR EVOKED POTENTIAL RECORDING IN HORSES USING INTRAMUSCULAR NEEDLE ELECTRODES AND SURFACE ELECTRODES

J. Rijckaert[1], B. Pardon[1], L. Van Ham[2], G. van Loon[1], P. Deprez[1]

[1] Department of Large Animal Internal Medicine, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, Merelbeke 9820, Belgium

[2] Small Animal Department, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, Merelbeke 9820, Belgium

Adapted from: Rijckaert, J., Pardon, B., Van Ham, L., van Loon, G., Deprez, P., 2018. Magnetic Motor Evoked Potential Recording in Horses Using Intramuscular Needle Electrodes and Surface Electrodes. Journal of Equine Veterinary Science 68, 101-107

ABSTRACT

To date, MEP recording in animals is often performed using intramuscular monopolar needle electrodes. Their placement and use has several disadvantages. Adhesive surface electrodes appear to be attractive since they are painless and easy to place. Since these are not used in horses a scouting study is performed to 1) explore the applicability of surface electrodes in horses 2) determine the repeatability of motor latency times (LAT) and amplitude measurements and 3) to investigate if LAT and amplitude values of surface electrode recordings were similar to intramuscular needle electrode recordings. Transcranial MMEP recordings were performed by both coated needle and surface electrodes on ten sedated warmblood horses. Mean LATs for the thoracic limbs were 20.8 ± 1.5 ms for needle and 21.2 ± 1.4 ms for surface electrode recording and 39.4 ± 3.8 ms and 39.2 ± 3.8 ms for the pelvic limbs, respectively. Mean amplitude values were 8.3 ± 4.1 and 7.2 ± 4.7 mV for the thoracic limbs and 4.2 ± 3.1 and 3.8 ± 2.4 mV for the pelvic limbs, respectively. A good agreement and repeatability for LAT but insufficient agreement and repeatability for amplitude between both recording types were determined by Bland-Altman plots and Passing-Bablok regression and coefficients of variation calculation. In conclusion, this preliminary study shows that surface electrode recording of MMEPs is possible and well tolerated in horses. Surface recordings were repeatable and look similar to the intramuscular recordings when regarding LATs, but overshadowing effects of large test-to-test variations precluded a conclusion concerning amplitude.

Key words: Ataxia, compound muscle action potential, electromyography, neurologic test, spinal cord

INTRODUCTION

To-date, non-invasive diagnostic testing of the motor function of the spinal cord is performed by recording of muscular motor evoked potentials (MEPs) that are elicited by transcranial magnetic stimulation (TMS) or transcranial electrical stimulation (TES). Mayhew *et al*. introduced the TMS technique in horses and obtained extra-muscular (e.m.) MMEPs at the surface of the skin (Mayhew and Washbourne, 1996). E.m. MMEPs are compound muscle potentials that reflect the electrical activity of many motorneurons. Subcutaneous (s.c.) needle electrodes measure similar e.m. MMEPs and are also applied in horses (Journée, 2014). Surface EMG (sEMG) is a non-invasive technique to measure summed muscle activity of many motorneurons on the skin overlying a muscle or group of muscles (Drost et al., 2006; Selvanayagam et al., 2012) and is widely used to record compound muscle action potentials.

Alternately, the group of Nollet *et al*. recorded transcranial elicited i.m. MMEPs in horses by inserting insulated needle electrodes with uncoated tips in muscles (Nollet et al., 2002; Nollet et al., 2004; Nollet et al., 2003a; Nollet et al., 2003c; Nollet et al., 2005). Intramuscular MMEPs result from a few single muscle fibers. Intramuscular needle electrodes are specific useful for diagnostics on the peripheral motorneuron function but is, when compared to s.c. needle or surface electrodes, very painful and therefore reason not to apply in children unless when strictly necessary (Drost et al., 2006). When compared to e.m.MMEPs, i.m.MMEPs will have variable and different amplitudes and more polyphasic waves since the characteristics of a only few lower motorneurons dominate the shape of the MMEPs (Hermens et al., 2000; Merletti and Farina, 2009). This means that surface and intramuscular electrodes are interchangeable for measuring motor latency times (LAT's).

Adhesive surface electrodes have successfully been used in electrocardiography in horses as alternative to alligator clips (Verheyen et al., 2010). Alligator clips have also been used for MMEP measurements with TMS (Mayhew and Washbourne, 1996). Adhesive surface electrodes offer features like being painless for the horse, absence of stick injuries, no risk of iatrogenic infections and being easy to apply.

To our knowledge, adhesive surface electrodes are not applied in horses for transcranial MMEP recording and may be an attractive alternative for intramuscular needle electrodes for measurement of only LAT's. This was reason for this scouting study to the characteristics and applicability of surface electrodes for recording of MMEPs that are elicited by TMS by comparing with intramuscular needle electrodes.

MATERIAL AND METHODS

All procedures were approved by the ethical committee (EC 2015/78).

SAMPLE SIZE CALCULATIONS AND ANIMALS

Sample size was calculated using Win Episcope 2.0. Sample size was estimated based on LAT (in milliseconds (ms)), since it is clinically the most decisive MMEP parameter. With a standard deviation (SD) of 1.8 ms in thoracic limbs and 2.8 ms in pelvic limbs (Nollet et al., 2002; Nollet et al., 2003b), 95% confidence and 80% power, 10 animals were required for a two-tailed test with paired samples.

Ten healthy horses (5 mares and 5 geldings; 7 warmbloods, 1 trotter, 1 Friesian and 1 Andalusian), aged between 3 and 17 years, (mean ± SD 11 ± 5 years) were used. Their height ranged from 153 to 169 cm (mean ± SD 160 ± 5 cm) and their weight from 460 to 660 kg (mean ± SD 535 ± 67 kg). Only horses without abnormalities on neurological examination (normal behaviour and mental status, head position and head movements, normal gait, posture and coordination) were included in this study.

MAGNETIC STIMULATION AND MMEP RECORDING

Each horse was sedated with a combination of detomidine (Domidine[1], 10 µg/kg bodyweight) and butorphanol (Dolorex[2], 10 µg/kg bodyweight). For each horse, magnetic motor evoked potentials (MMEPs) recording for i.m. needle and surface electrodes was done in one single sedation period. The test protocol started in 5 horses with i.m. needle electrodes and in the remaining 5 horses with surface electrodes.

A magnetic stimulator (Magstim 200)[3] and a round 70 mm coil were used to generate a maximal magnetic field of 4 Tesla at the coil surface. The coil was centred over the forehead and maximal stimulus intensity (100%) was applied (Nollet et al., 2004). A standard electromyograph (Medelec Sapphire)[4] recorded the muscle responses. For MMEP recording with needle electrodes, the procedure as described by Nollet *et al.* (Nollet et al., 2004) was followed. The active electrode (25 mm monopolar, disposable, insulated, stainless steel needle)[5] was inserted at the middle of the tibialis cranialis muscle in the pelvic limbs and of the extensor carpi radialis muscle in the thoracic limbs. The reference electrode was placed subcutaneously, at the lateral side of the lateral malleolus of the tibia for the pelvic limb and at the lateral side of the radial tuberosity for the thoracic limb. Following the recommendation of Verheyen et al. (2010) for recording of ECG on horses, Skintact FS50[6] adhesive surface electrodes were attached to the unclipped skin. The first electrode was placed, analogous to the needle electrode, at the middle of the tibialis cranialis muscle (TC) in the pelvic limbs and the extensor carpi radialis muscle (ECR) in the thoracic limbs. The second electrode was placed at the central part of the distal tendon of the corresponding muscle (Figure 1). For both recording types, the ground electrode was attached in the groin region while testing the pelvic limbs and in the elbow region while testing the thoracic limbs. For every limb, 4 sequential muscle responses were recorded starting with the left pelvic and the right pelvic limb followed by the left thoracic and finally the right thoracic limb. Thereafter, the test was immediately repeated with the other electrode type, resulting in 32 recordings per horse.

Of each elicited MMEP, LAT and amplitude were acquired as characterizing parameters. The LAT is defined as the time interval between the onsets of the TMS pulse and MMEP wave and measured in millisecond (ms) units. The amplitude was measured as the difference between the largest peaks of opposite polarity and measured in millivolt (mV) units. After completion of the 32 MMEP measurements per horse, the MMEP parameters were analysed from MMEP curves on the screen. All curves were archived as printed screen copies. All measurements and MMEP analysis were performed by one non-blinded operator.

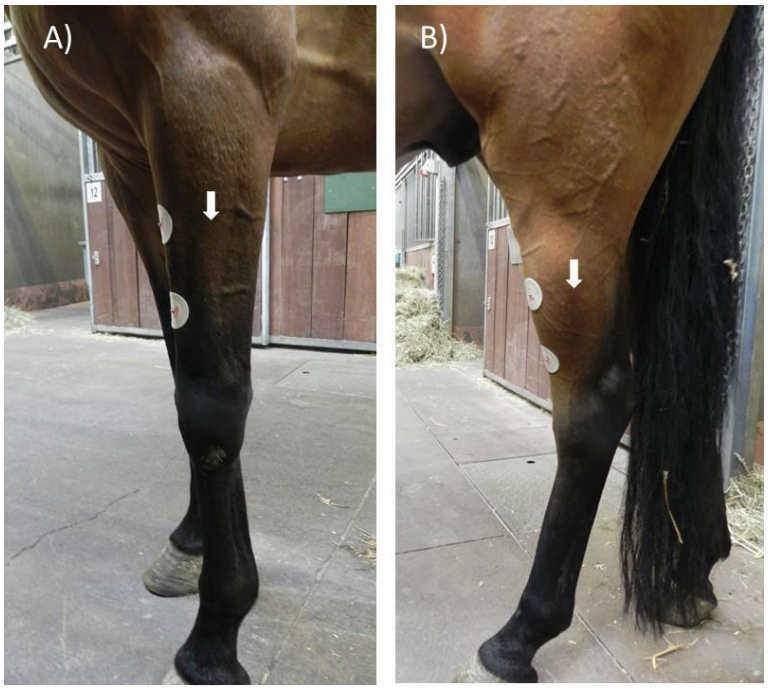


Figure 1. Positioning of the surface electrodes in thoracic (A) and pelvic (B) limbs. The first needle electrode was placed at the level of the proximal surface electrode, the arrow indicates the position of the second needle electrode.

STATISTICAL ANALYSIS

All responses were included for statistical analysis. Means, SD, minimum, maximum, mean difference, minimum difference, maximum difference and 95% confidence intervals (CI) of LAT and amplitude recording with both methods were calculated. To determine repeatability for both methods, the 4 responses per limb were superposed on the EMG screen and coefficients of variation (CV (%)= (SD)/mean * 100) were calculated on limb and estimated CVs (estimated CV = $(1+(4 * \text{number of observation})^{-1}) * CV$) on horse level. CV on limb level is the mean of the thoracic and pelvic limb CVs for each horse. CV on horse level was calculated using the minimum values for LAT and the maximum values for amplitude. The tests with the lowest CVs have the best repeatability.

Subsequently, Passing-Bablok regression was used to compare MMEP recording with both electrode types. This non-parametric test determines a regression equation (Y=a +bX) between recordings of the same subject with two recording methods, with Y being MMEP recording with surface electrodes and X recording with needle electrodes. No systematic differences are present if the 95% CI of the intercept (a) contained 0. No proportional differences are present if the 95% CI of the slope (b) contains 1. Confirmation of a linear relationship between both methods was assessed with a cumulative sum control (CUSUM) test. All analyses were performed in Microsoft Excel 2016.

RESULTS

In 10 horses, a total of 320 stimuli in thoracic and pelvic limbs were done. All stimulations led to measurable responses. The needles were in general more difficult to place than the surface electrodes and lost their position occasionally (2-3 times per 16 stimuli) because of mild reactions (muscle trembling, moving of the limbs,…) of the horses. During a test run with a series of 4 subsequent TMS stimuli, the change in electrode position was recognized by varying MEP wave forms starting with a typical intramuscular polyphasic morphology that sometimes transited into extramuscular MEP wave patterns resembling those of surface electrodes with a decreased number of phases. Figure 2 shows a typical polyphasic intramuscular MMEP (left) and a typical MMEP of surfaces electrodes with a reduced number of phases (right). Surface electrodes did not dislodge during the 4 runs and their superimposed MMEPs showed good reproducible wave shapes when compared to the 4 runs superimposed i.m.MMEPs, often showing large varying wave patterns.

Means, SD, minimum, maximum, 95% CI and CV of LAT and MMEP amplitude for both electrode types are shown in Table 1. For LAT, the mean difference between needle and surface electrode recordings was -0.4 ms (minimum -2.6 ms, maximum 2.7 ms) in the thoracic limbs and 0.1 ms (minimum -9.5 ms, maximum 6.5 ms) in the pelvic limbs. For MMEP amplitude these values were respectively 1.1 mV (minimum -11.6, maximum 14.7 mV) and 0.4 mV (minimum -9.1mV, maximum 13.4 mV).

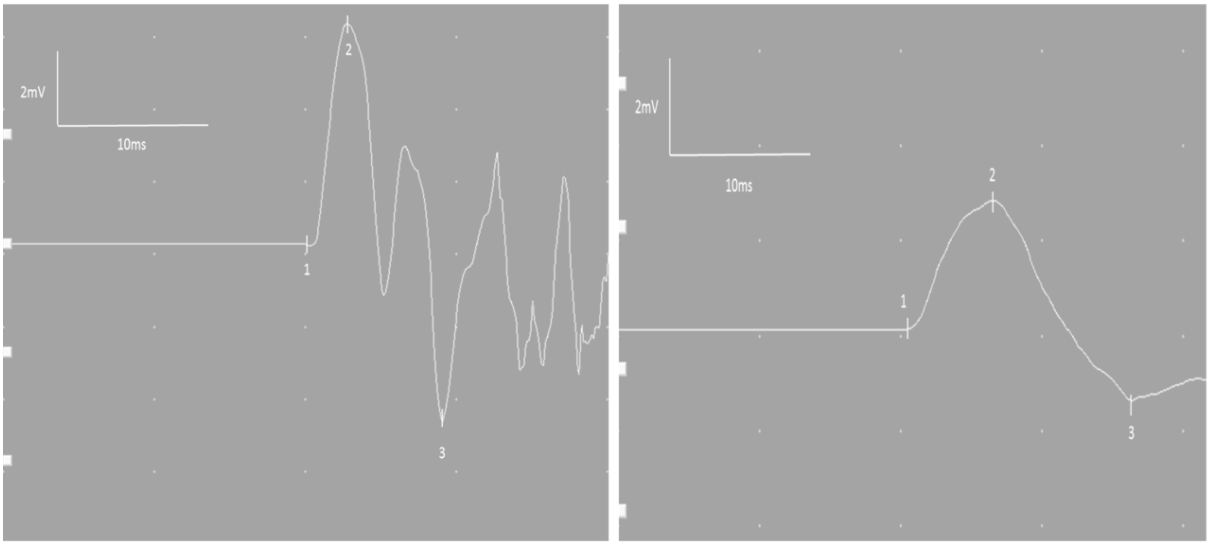


Figure 2. Example of a MMEPs recorded in the thoracic limb with needle electrodes (left) and surface electrodes (right). The time between Y-axis and number 1 reflects LAT, the amplitude is measured between numbers 2 and 3.

Table 2 and Figure 3 show Passing-Bablok regression graphs and equations for LAT and MMEP amplitude in thoracic and pelvic limbs. No systematic or proportional differences between needle and surface electrode recording for LAT were present in thoracic or pelvic limbs. For MMEP amplitude, no systematic or proportional differences were present in the pelvic limbs, but there were systematic differences recorded in thoracic limbs. For all recordings, except MMEP amplitude in the pelvic limbs, CUSUM test confirmed linearity.

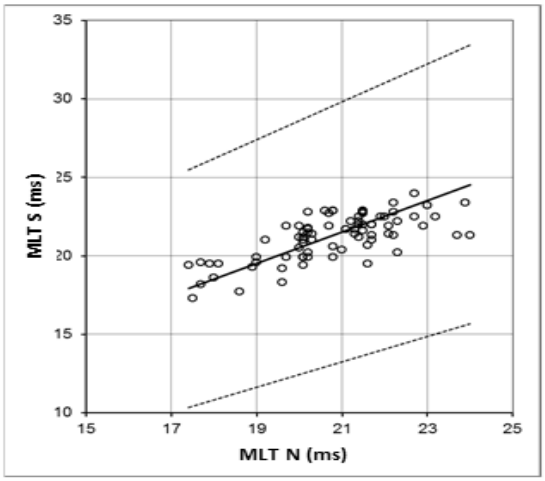
Table 1. Comparison of LAT and MMEP amplitude measurements with needle (LAT N and AMPL N) and surface (LAT S and AMPL S) electrodes (CI: confidence interval, CV: coefficient of variation).

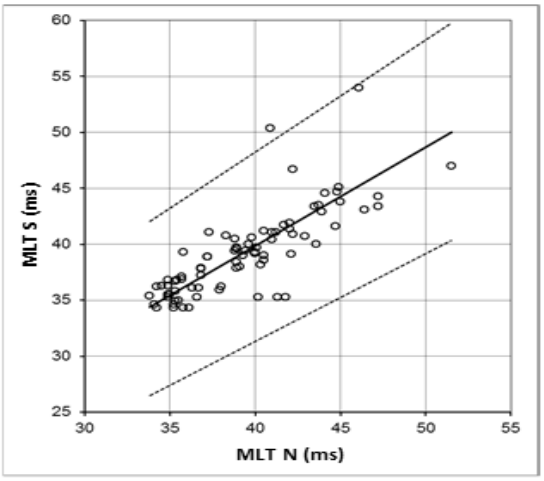
	LAT N (ms)		LAT S (ms)		AMPL N (mV)		AMPL S (mV)	
Limb	Thoracic	Pelvic	Thoracic	Pelvic	Thoracic	Pelvic	Thoracic	Pelvic
Mean	20.8	39.4	21.2	39.2	8.3	4.2	7.2	3.8
SD	1.5	3.8	1.4	3.8	4.1	3.1	4.7	2.4
Minimum	17.4	33.8	17.3	34.3	0.4	0.2	1.1	0.8
Maximum	24.0	51.5	24.0	54	18.6	15.6	17.7	9.7
95% CI	20.5-21.1	38.5-40.2	20.9-21.5	38.4-40.1	7.4-9.2	3.5-4.9	6.1-8.3	3.3-4.4
Mean CV limb	3.0 %	3.5 %	3.2%	3.3%	35.0%	59.6%	32.6%	39.5%
SD of mean CV limb level	1.73%	1.68%	1.25%	2.59%	6.96%	12.65%	22.40%	12.71%
Estimated CV horse level	8.4%	9.0%	7.5%	7.9%	22.8%	23.8%	27.5%	28.5%

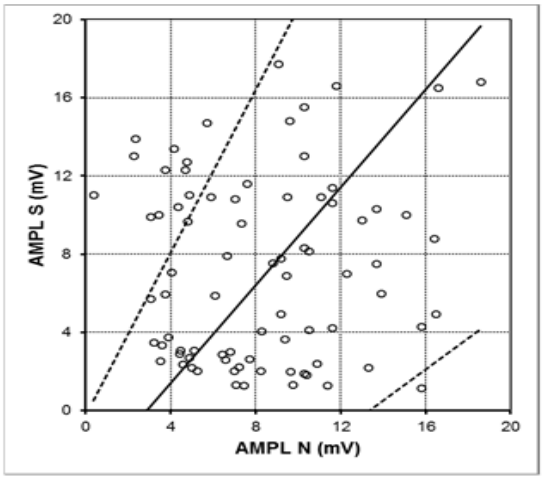
Table 2. Passing-Bablok regression equation with 95% confidence interval for intercept and slope and *P*-values for CUSUM test for LAT and amplitude in thoracic and pelvic limbs. (LAT S: LAT recorded with surface electrodes, LAT N: LAT recorded with needle electrodes, AMPL S: amplitude recorded with surface electrodes, AMPL N: amplitude recorded with needle electrodes, CI: confidence interval, CUSUM: cumulative sum control)

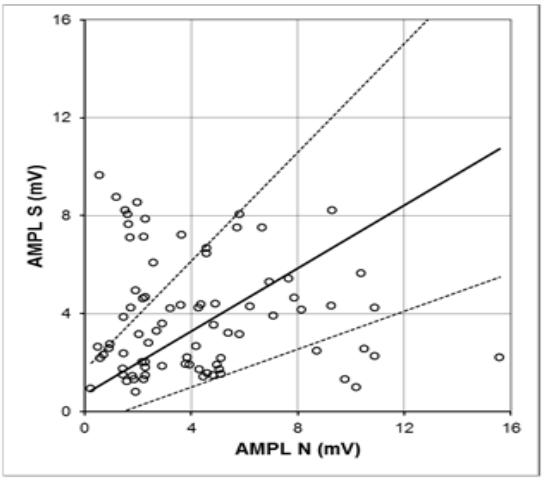
Parameter	**Thoracic/Pelvic limbs**	**Regression**	**95% CI intercept**	**95% CI slope**	***P*-value CUSUM**
LAT	**Thoracic**	LAT S = 0.6 + 1.0 LAT N	(-3.7 ; 4.6)	(0.8 ; 1.2)	0.80
	Pelvic	LAT S = 4.5 + 0.9 LAT N	(-0.1 ; 8.2)	(0.8 ; 1.0)	0.80
Amplitude	**Thoracic**	AMPL S = -3.6 + 1.3 AMPL N	(-10.7 ; -0.2)	(0.8 ; 2.1)	0.80
	Pelvic	AMPL S = 0.7 + 0.6 AMPL N	(-0.5 ; 1.7)	(0.4 ; 1.1)	0.20

Figure 3. Passing-Bablok plot of LAT and amplitude recorded with surface electrodes (LAT S and AMPL S) and needle electrodes (LAT N and AMPL N) for thoracic limbs (left) and pelvic limbs (right).









DISCUSSION

Adhesive surface electrodes have successfully been used in electrocardiography (Verheyen et al., 2010). Their features invited us to introduce these in horses and use them for assessment of the motor function of spinal cord function using TMS. Currently most TMS-MMEP tests are performed in horses with intramuscular needle electrodes and used to diagnose impaired motor functions of the spinal cord in horses with clinical signs of impaired motor function, like ataxia, muscle weakness, spasticity, dystonia, abnormal reflexes and myopathy, resulting from lesions in the spinal cord, brain stem and brain (Nollet et al., 2002; Nollet et al., 2004; Nollet et al., 2003c; Nollet et al., 2005). The goal of this pilot study is to explore the features of the use of adhesive surface electrodes and to compare these with intramuscular electrodes.

The practical features of self-adhesive surface electrodes in ECG recordings in horses apply also to TMS. Self-adhesive electrodes are well tolerated while clipping of the hair coat is generally not necessary or even discouraged (Verheyen et al., 2010). The electrodes are painless, non-invasive and easy to mount. All TMS stimuli resulted in detectable MMEPs in all recorded muscle groups and did not dislodge. In contrast, the i.m. needle electrodes were in general more difficult to place and migrated from their location about 2-3 times per 16 stimuli due to elicited or spontaneous muscle movements and trembling of the horses. This could be explained by unequal displacements of the muscle and overlaying skin during contractions. Since i.m. needle electrodes are mechanical connected with both tissues, large muscle excursions may cause gradual electrode dislocations. When not sedated, inserting i.m. electrodes in muscles can be very painful (Drost et al., 2006). The mechanical disturbance from inserting the electrode in the muscle may also affect the mechanical receptors in afferents of extrafusal fibers initiating spinal reflexes and may cause muscle trembling. Based on the fact that the horses reacted less on the placement of the surface electrodes when compared to i.m. needle electrode placement, it can be stated that the first are better tolerated.

In this study, the characteristics of i.m. MMEPs are compared with MMEPs from surface electrodes. This implies an important limitation on the set-up of the present study. Surface electrodes and intramuscular needle electrodes belong to different classes of respectively extramuscular end intramuscular EMG recording. The e.m. class embrace electrode locations outside muscles: on the skin and subcutaneous. Comprised are electrode types on the skin

like surface- and alligator clip electrodes and s.c. electrodes at subcutaneous level. Insulated needle electrodes with uncoated tips belong to the i.m.class. I.m. needle electrodes are sensitive for the electrical activity of muscle fibers of only a few motorneurons (Merletti and Farina, 2009). These are predominantly used to evaluate the lower motor unit function. This means that in transcranial stimulation, i.m.MMEPs only senses a relative small fraction of all activated motorneurons. A typical polyphasic MMEP wave from TMS as recorded by a coated i.m. electrode is shown in Figure 2a. EMG electrodes of the e.m. class are sensitive to the electrical activity of many muscle fibers of a whole muscle, sometimes extending to neighbour muscles (Selvanayagam et al., 2012; Summers et al., 2017). In the bipolar arrangement e.m.electrodes measure the sum of action potentials representing the whole muscle activity and even of neighbour muscles (Hermens et al., 2000). The specific motor unit potentials subsides due to phase cancelation of action potentials of individual muscle fibres. Intramuscular recordings have a higher number of turns and higher frequencies than surface electrode recordings (Bolzoni et al., 2013). A typical e.m.MMEP wave form as obtained from surface electrode is shown in Figure 2b. The number of phases is clearly reduced compared to Figure 2a.

One shortcoming in the setup of this study is that a comparison between MMEPs from surface and intramuscular electrodes not specific looks at different characteristics of electrode types only. Characteristics from both electrode classes are also included. This complicates a comparison. The electrode location predicts differences in MMEP wave shape and amplitude, while LATs are expected to be about the equal to each other. The e.m.- i.m. bias between classes would be eliminated when electrodes in the same class are compared. A comparison of surface electrodes with s.c.needle electrodes, both belonging to the e.m. class, would be more appropriate. Their EMGs of spontaneous activity and wave shapes of MMEPs are highly coherent, which is not the case with i.m. coated needle electrode EMGs (Skinner et al., 2008).

COMPARISON OF LATS

It was hypothesized that when nerve action potentials arrive synchronous at neuromuscular zones, e.m. and i.m. motor latency times (LAT) are statistically equal to each other. According to table 1, this study shows an agreement between LATs with coated i.m.needle and surface electrodes in horses when looking to overall means. Table 1 shows largely overlapping LATs for surface and i.m.needle electrodes for the ECR: 20.8 ± 1.5 ms and 21.2 ± 1.4 ms, and for TC: 39.4 ± 3.8 ms and 39.2 ± 3.8 ms. The mean values of both electrode types show no statistical differences. The overall differences of 0.2 and 0.4 ms supports the hypothesis that LATs from surface and the i.m.needle electrodes are equal to each other. The low CVs indicated good and acceptable intra-individual (within horses) and inter-individual (between horses) reproducibility of repeated MMEP measurements for each electrode type on the thoracic and pelvic limb muscles. Furthermore, no systematic or proportional differences between needle and surface electrode recording were found using Passing-Bablok regression.

The literature provides normal data for LATs for extra- and intramuscular MMEPs that support our data. Mayhew et al started at first in 1996 with e.m. EMG recording on the skin surface using alligator clips (Mayhew and Washbourne, 1996) and assessed 10 healthy pony's with TMS. Mean LATs and standard deviation were for the ECR: 19.0 ± 2.3 ms and for the TC: 30.2 ± 3.4 ms. LATs of subdermal needle electrodes in 12 healthy horses using TES were for left and right ECR: 20.8 ± 1.85 ms and 19.7 ± 1.69 ms and TC: 34.6 ± 2.01 and 34.9 ± 1.69 ms (Journée, 2014). The group of Nollet *et al*. measured TMS-MMEPs with i.m.needle electrodes. One paper reveals in 12 healthy horses LATs for left and right for ECR: 20.81± 1.85 ms and 20.59 ± 1.83 and for TC: 35.94 ± 3.43 and 36.33 ± 3.53 (Nollet et al., 2002). Another study of this group on 84 horses reveals LATs for the ECR: 19.32 ± 2.5 ms and for the TC: 30.54 ± 5.28 ms (Nollet et al., 2004). This last mean LAT value is 4-6 ms lower than in their other study and also in the other two e.m.MMEP studies (Journée, 2014; Mayhew and Washbourne, 1996). However, the mean LAT values for the pelvic muscles in our study are even 4 to 9 ms higher than i.m.LATs of the group of Nollet. This cannot be explained by differences in height of included horse groups (Nollet et al., 2004). The higher means are explained by about 15% of the points in the upper-right scatter-plot of the pelvic limb that exceed the range of normal values of all referred papers. These values comply with data of two horses with bilateral hind limb ataxia showing only slightly prolonged LATs near the 95% CI of normal values (Nollet et al., 2003c).

Since no myelograms are available, a subclinical myelopathy cannot be excluded in a few horses in this study.

Individual differences of LATs between the two electrode types are visualized in the scatter diagrams of the LATs of the thoracic and pelvic limbs. The width of the point clouds around the Passing-Bablok regression lines (parameters are listed in table 2) indicate the variation between LATs of the two electrode types. The variation in the upper left plot of the thoracic muscles is about ± 3 ms and in the upper right plot of the pelvic muscles is, except for 2 outliers, about ± 5 ms. When including all points, the range of differences for the pelvic muscle group is 2.6 – 9.9 ms. These are high values and essentially different from literature data where simultaneous measured MMEPs of both electrodes are assessed. LAT differences between intramuscular and surface electrodes are one magnitude lower in a sub-millisecond range (Verin et al., 2002). The variations of our study mainly reflect test-to-test variations of LATs due to spontaneous varying spinal facilitation that modulate motor neuron membrane potentials. When increasing the facilitation, the LAT of TMS-MMEPs of striated muscles of both surface and i.m. electrode types the LATs decrease by 2-3 ms (Brostrom et al., 2003; Kaneko et al., 1996). Repositioning of the magnetic coil may also contribute to test-to-test variations (Kaneko et al., 1997; Kaneko et al., 1996). It is concluded that the set-up of this study is insufficient to assess LAT differences of transcranial elicited MMEPs between intramuscular and surface electrodes individually due to the overshadowing by relative large test-to-test variations.

COMPARISON OF MMEP AMPLITUDES.

Table 1 shows a large overlap of the 95% CI of the MMEP amplitudes of i.m. needle and surface electrodes for the thoracic limb muscles of respectively 7.4-9.2 and 6.1-8.3 mV and for the pelvic limb muscles 3.5 - 4.9 and 3.3 - 4.4 mV. The maximum differences ranged from -9.1 to 14.7 mV. The mean amplitudes of both electrode types are statistically not different. The high CVs and high SD values express high intra-individual test-to-test variations of muscle MMEP amplitudes and inter-individual differences between horses. High test-to-test variations are also reported in transcranial MMEP studies in horses (Journée, 2014; Nollet et al., 2002; Nollet et al., 2003a; Nollet et al., 2003b; Nollet et al., 2003c). Individual differences of MMEP

amplitudes between the two electrode types are visualized in the scatter diagrams of the thoracic and pelvic limbs in Figure 3. The wide-spread point clouds of the plots at the bottom in around the Passing-Bablok regression lines (parameters are listed in table 2) indicate the large variation between LATs of the two electrode types. The variation is dominated by the test-to-test amplitude variations. These are responsible for the wide scatter in the plots of the MMEP amplitudes of the surface and i.m. needle electrodes. A linear relationship is ruled out in the CUSUM test. However, the dominating high test-to-test variations mask any possible relationship between amplitudes of alternate recorded MMEPs from i.m. needle and surface electrodes. Also here, it is concluded that the set-up of this study, that is based on sequential comparison, precludes assessment amplitude differences of transcranial MMEPs between i.m. and surface electrodes due to the overshadowing by relative large test-to-test variations. It is therefore impossible to check the hypothesis that LAT differences of both electrode types are equal to each other and that MMEPs amplitudes probably are unrelated. Test-to-test influences can be ruled-out in a study set-up that is based on pairwise comparison on simultaneous recorded MMEPs of both electrode types.

In human, the impedance of surface electrodes is usually higher than needle electrodes. High impedances may increase the background noise which may mask small sMMEPs in deteriorated spinal cord functions. Interposed unclipped hair could possibly augment the impedance of surface electrodes. The range of sMMEP amplitudes of normal horses is in a range of 1 to over 10mV. This is large enough by which surface electrode impedances are not of a concern. A remaining unanswered question is how critical the impedance is for detectability of small sMMEPs in elevated background noise at spinal cord lesions.

RECAPITULATION.

This pilot study indicates that surface and intramuscular insulated i.m. electrodes both are useful to assess the motor function of the spinal cord by assuming the LAT as most important diagnostic parameter. The very small differences between the mean values of LATs of both electrode types supports the hypothesis that both electrode types deliver equal latency times. However, sensitivity to the test-to-test variations of the used study method precludes the possibility to precisely quantify the difference between LATs of pairwise recorded transcranial

MMEPs at each test. This should be elaborated in a subsequent study with simultaneous MMEP recording by the two electrode types placed on the same locations on the skin and intramuscular. When the differences per stimulus are indeed within a sub-milliseconds size, then i.m. needle electrodes and surface electrodes can be interchanged while normative data of LATs can be shared by both electrode types. Besides practical features of easy, non-invasive and painless placement, surface electrodes sense larger portions of activated motorneurons while the sensitivity for individual motor units that harbour lower motor neuron system functions, for which i.m. electrodes are designed, is suppressed (Skinner et al., 2008). This is in favour for the selectivity and sensitivity for spinal motor function.

LIMITATIONS OF THE STUDY.

1. Test-to-test variations of LAT and MMEP amplitudes mask true within single tests differences of LATs and MMEP amplitudes between electrode types due to sequential comparison. These variations could be excluded by pairwise comparison of simultaneous recorded MMEPs of both electrode types.

2. Specific characteristics of electrode types are intermingled with the recording properties from extra and intramuscular locations. Comparison with subdermal needle electrodes would more specific expose the qualities of adhesive surface electrodes.

3. Lack of knowledge of noise levels from unknown impedances of surface electrodes leaves an open question on detectability of small MMEPs in impaired motor functions of the spinal cord in comparison with concurrent needle electrodes.

4. Non-blinded data assessment by one observer.

CONCLUSION

This preliminary study indicates that the adhesive surface electrodes add a value in equine neurology studies. Besides practical features as easy placement, painless and non-invasive, they do not dislodge during muscle movements and are sensitive for a large portion of activated motorneurons. The study shows that mean values of LATs from surface and i.m. coated needle electrodes are equal to each other while thoracic limb LATs comply with normal data of MMEPs from e.m. and i.m. MMEPs in literature. A supplementary study based on simultaneous recording sMMEPs and i.m.MMEPs and pairwise comparison is necessary for appropriate validation of LAT and amplitude differences within individual tests.

REFERENCES

Bolzoni, F., Pettersson, L.G., Jankowska, E., 2013. Evidence for long-lasting subcortical facilitation by transcranial direct current stimulation in the cat. The Journal of physiology 591, 3381-3399.

Brostrom, S., Jennum, P., Lose, G., 2003. Motor evoked potentials from the striated urethral sphincter: a comparison of concentric needle and surface electrodes. Neurourology and Urodynamics 22, 123-129.

Drost, G., Stegeman, D.F., van Engelen, B.G., Zwarts, M.J., 2006. Clinical applications of high-density surface EMG: a systematic review. Journal of electromyography and kinesiology : official journal of the International Society of Electrophysiological Kinesiology 16, 586-602.

Hermens, H.J., Freriks, B., Disselhorst-Klug, C., Rau, G., 2000. Development of recommendations for SEMG sensors and sensor placement procedures. Journal of electromyography and kinesiology : official journal of the International Society of Electrophysiological Kinesiology 10, 361-374.

Journée, S.L., Delesalle, C.J.G., de Bruijn, C.M., Bergmann, W. and Journée, H.L., 2014. Transcranial electrical stimulation (TES) as a possible novel alternative to transcranial magnetic stimulation (TMS) to assess the motor function of the spinal cord for clinical diagnosis in horses. Equine Veterinary Journal 46, Suppl. 47, 10.

Kaneko, K., Fuchigami, Y., Morita, H., Ofuji, A., Kawai, S., 1997. Effect of coil position and stimulus intensity in transcranial magnetic stimulation on human brain. Journal of the neurological sciences 147, 155-159.

Kaneko, K., Kawai, S., Fuchigami, Y., Shiraishi, G., Ito, T., 1996. Effect of stimulus intensity and voluntary contraction on corticospinal potentials following transcranial magnetic stimulation. Journal of the neurological sciences 139, 131-136.

Mayhew, I.G., Washbourne, J.R., 1996. Magnetic motor evoked potentials in ponies. Journal of Veterinary Internal Medicine 10, 326-329.

Merletti, R., Farina, D., 2009. Analysis of intramuscular electromyogram signals. Philosophical transactions. Series A, Mathematical, physical, and engineering sciences 367, 357-368.

Nollet, H., Deprez, P., Van Ham, L., Verschooten, F., Vanderstraeten, G., 2002. The use of magnetic motor evoked potentials in horses with cervical spinal cord disease. Equine Veterinary Journal 34, 156-163.

Nollet, H., Deprez, R., Van Ham, L., Dewulf, J., Decleir, A., Vanderstraeten, G., 2004. Transcranial magnetic stimulation: normal values of magnetic motor evoked potentials in 84 normal horses and influence of height, weight, age and sex. Equine Veterinary Journal 36, 51-57.

Nollet, H., Van Ham, L., Dewulf, J., Vanderstraeten, G., Deprez, P., 2003a. Standardization of transcranial magnetic stimulation in the horse. Veterinary Journal 166, 244-250.

Nollet, H., Van Ham, L., Gasthuys, F., Dewulf, J., Vanderstraeten, G., Deprez, P., 2003b. Influence of detomidine and buprenorphine on motor-evoked potentials in horses. Veterinary Record 152, 534-537.

Nollet, H., Van Ham, L., Verschooten, F., Vanderstraeten, G., Deprez, P., 2003c. Use of magnetic motor-evoked potentials in horses with bilateral hind limb ataxia. American Journal of Veterinary Research 64, 1382-1386.

Nollet, H., Vanschandevijl, K., Van Ham, L., Vanderstraeten, G., Deprez, P., 2005. Role of transcranial magnetic stimulation in differentiating motor nervous tract disorders from other causes of recumbency in four horses and one donkey. Veterinary Record 157, 656-658.

Selvanayagam, V.S., Riek, S., Carroll, T.J., 2012. A systematic method to quantify the presence of cross-talk in stimulus-evoked EMG responses: implications for TMS studies. Journal of applied physiology 112, 259-265.

Skinner, S.A., Transfeldt, E.E., Savik, K., 2008. Surface electrodes are not sufficient to detect neurotonic discharges: observations in a porcine model and clinical review of deltoid electromyographic monitoring using multiple electrodes. The Journal of Clinical Monitoring and Computing 22, 131-139.

Summers, R.L., Chen, M., Kimberley, T.J., 2017. Corticospinal excitability measurements using transcranial magnetic stimulation are valid with intramuscular electromyography. PloS one 12, e0172152.

Verheyen, T., Decloedt, A., De Clercq, D., Deprez, P., Sys, S.U., van Loon, G., 2010. Electrocardiography in horses - part 1: how to make a good recording. Vlaams Diergeneeskundig Tijdschrift 79, 331-336.

Verin, E., Straus, C., Demoule, A., Mialon, P., Derenne, J.P., Similowski, T., 2002. Validation of improved recording site to measure phrenic conduction from surface electrodes in humans. Journal of applied physiology 92, 967-974.

CHAPTER 6

DETERMINATION OF MAGNETIC MOTOR EVOKED POTENTIAL LATENCY TIME CUT-OFF VALUES FOR DETECTION OF SPINAL CORD DYSFUNCTION IN HORSES

DETERMINATION OF MAGNETIC MOTOR EVOKED POTENTIAL LATENCY TIME CUT-OFF VALUES FOR DETECTION OF SPINAL CORD DYSFUNCTION IN HORSES

J. Rijckaert. [1], B. Pardon. [1], V. Saey. [2], E. Raes. [3], L. Van Ham [4], R. Ducatelle [2], G. van Loon [1], P. Deprez[1].

[1] Department of Large Animal Internal Medicine, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, Merelbeke 9820, Belgium

[2] Department of Pathology, bacteriology and poultry diseases, Ghent University, Salisburylaan 133, Merelbeke 9820, Belgium

[3] Department of Veterinary medical imaging and small animal orthopedics, Ghent University, Salisburylaan 133, Merelbeke 9820, Belgium

[4] Small Animal Department, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, Merelbeke 9820, Belgium

Adapted from: Rijckaert, J., Pardon, B., Saey, V., Raes, E., Van Ham, L., Ducatelle, R., van Loon, G., Deprez, P., Determination of magnetic motor evoked potential latency time cut-off values for detection of spinal cord dysfunction in horses. Journal of Veterinary Internal Medicine. (Accepted June 2019).

ABSTRACT

Background - Transcranial magnetic stimulation (TMS) and recording of magnetic motor evoked potentials (MMEPs) is a valuable tool to detect neurological dysfunction in horses. Increased latency times of MMEPs and ataxia show a good association, but cut-off values based on confirmed spinal cord dysfunction, are lacking.

Objectives - Latency time cut-off determination for neurological dysfunction detection.

Animals - Five control horses and 17 horses with ataxia

Methods – Case control study with receiver operating characteristic (ROC)-curve analysis, based on diagnostic imaging, TMS and histopathological findings.

Results - Diagnostic imaging and histopathology did not show abnormalities in the control group but confirmed spinal cord compression in 14/17 ataxic horses. In the remaining 3 horses histopathological lesions were mild to severe, but diagnostic imaging did not confirm spinal cord compression. In control horses, latency time values of thoracic and pelvic limbs were significantly lower than in ataxic horses (20±1 versus 34±16 ms, *P*=0.048 and 39±1 versus 78±26 ms, *P*=0.0042). Optimal cut-off values to detect spinal cord dysfunction were 22 ms (sensitivity (95% Confidence interval (CI))=88% (73-100%), specificity (95% CI)=100% (100-100%)) in thoracic and 40 ms (sensitivity=94% (83-100%), specificity=100% (100-100%)) in pelvic limbs. To detect spinal cord dysfunction caused by compression, the optimal cut-off for thoracic limbs remained 22 ms while it increased to 43 ms in pelvic limbs (sensitivity=100% (100-100%), specificity=100% (100-100%) for thoracic and pelvic limbs).

Clinical relevance - MMEP analysis using these cut-off values appears to be a very promising diagnostic tool for detection of spinal cord dysfunction in horses

Keywords: Ataxia, Cervical radiographs, Cervical vertebral malformation, Myelogram, Transcranial magnetic stimulation

INTRODUCTION

In warmblood horses (Levine et al., 2008; Levine et al., 2010; Szklarz et al., 2018; Walmsley, 2005), Tennessee walking horses (Levine et al., 2008; Levine et al., 2010) and thoroughbreds (Levine et al., 2008; Levine et al., 2010; Oswald et al., 2010) spinal ataxia is common. Major causes include cervical vertebral malformation (CVM), equine degenerative myeloencephalopathy and neuroaxonal dystrophy (EDM/NAD), equine protozoal myeloencephalitis (EPM) and trauma (Nout and Reed, 2003). EPM and EDM/NAD are important causes in the USA (Hamir et al., 1992) but worldwide, the most common cause probably is CVM (Nappert et al., 1989; Tyler et al., 1993). CVM, also called cervical vertebral stenotic myelopathy (CVSM), cervical vertebral compressive myelopathy (CVCM) or Wobblers' syndrome, causes dynamic or static compression of the spinal cord (Nout and Reed, 2003). The dynamic form is predominantly seen in young, fast growing thoroughbreds and warmblood horses (Levine et al., 2007; Levine et al., 2008; Levine et al., 2010) while the static form is more common in older horses (Levine et al., 2007; Nout and Reed, 2003; Oswald et al., 2010). A correct diagnosis of the exact etiology of ataxia is important, as different causes imply different treatment options and prognosis. However, diagnosis of the specific etiology remains challenging and mostly a combination of techniques is necessary.

The neurological examination of the ataxic horse often already indicates the gross localization of the lesion to an experienced examiner. Subsequently, diagnostic imaging of the suspected region is used to identify the causative lesion or to rule in or rule out differential diagnoses. However, because of the size of the horses, the spinal canal can be difficult to evaluate in detail, especially in the thoracic and lumbar region. Furthermore, sensitivities (43-85%) and specificities (70-78%) of plain radiographs or myelograms are too low for adequate diagnosis of spinal cord compression (Levine et al., 2007; Levine et al., 2010) and variation between measurements and observers can result in discrepancies in diagnosis (Hughes et al., 2014). CT and MRI are sensitive techniques but still do not provide information about the function of the nervous system. Furthermore, CT scans with a gantry large enough to visualize C6 and C7 in adult horses are expensive and not everywhere available and MRI scans are only able to scan the cranial part of the neck, currently. Therefore, often the diagnosis of CVM needs to be confirmed post-mortem by histopathology.

A supplementary, non-invasive diagnostic test for CVM, could be transcranial magnetic stimulation (TMS) with recording of magnetic motor evoked potentials (MMEPs). TMS is standardized in horses using a 70mm round coil to produce a maximal output of 4 Tesla on the upper medial forehead of the horse (Nollet et al., 2003a). The magnetic stimulation excites the descending motor tracts and the resulting muscle contraction (MMEP) is registered at the tibialis cranialis in the pelvic and the extensor carpi radialis muscle in the thoracic limbs (Nollet et al., 2004; Nollet et al., 2003a; Rijckaert et al., 2018). The time between stimulation and muscle contraction is called the latency time and is the most important parameter. The peak-to-peak amplitude of MMEP is a second parameter, but shows a lot of variation, even in normal horses (Nollet et al., 2004; Nollet et al., 2003b; Rijckaert et al., 2018) and is therefore more difficult to use in practice. Left or right side measurements, coil current (Nollet et al., 2004; Nollet et al., 2003a), sex, age (Nollet et al., 2004), sedation with detomidine and butorphanol (Nollet et al., 2003b) and the surface of needle electrode recordings (Rijckaert et al., 2018) do not have a significant influence on MMEP latency time. Weight, wither height and length do influence latency time in normal horses.

As mentioned above, TMS assesses the conduction through the descending pathways and thus the motor function of the spinal cord (Nollet et al., 2002; Nollet et al., 2003c; Nollet et al., 2005). The proprioceptive, ascending pathways cannot be evaluated with TMS, while ataxia is by definition a lack of proprioception. However, in cases of ataxia, very often both ascending (proprioceptive) and descending (motor) pathways are affected. Therefore, TMS can be helpful in these cases, showing prolonged latency times (Nollet et al., 2002; Nollet et al., 2003c). In horses with thoracic or thoracolumbar pathology, only pelvic limb MMEPs are abnormal. In cases with cervical spinal cord disease, both thoracic and pelvic MMEP values are clearly prolonged (Nollet et al., 2002). Latency times in ataxic horses range from 28 to more than 200 ms in the thoracic and from 53 to more than 200 ms in the pelvic limbs (Nollet et al., 2002; Nollet et al., 2003c), but in most of these studies there is no confirmation of the exact etiology by histopathology or diagnostic imaging. In dogs, there is a correlation between the severity of the neurological deficits and MMEP latency time (da Costa et al., 2006; Martin-Vaquero and da Costa, 2014; Poma et al., 2002). In cases with mild or subclinical neurological deficits, latency times might be only slightly prolonged. Therefore, reliable cut-off values are required to help clinicians declaring an animal healthy or affected, with the highest certainty

possible. However, in horses, no cut-off values have been determined yet. Therefore, the objective of this study was to determine MMEP latency time cut-off values for detection of spinal cord dysfunction in horses with determination of the cause of spinal cord dysfunction through diagnostic imaging and histopathology.

MATERIALS AND METHODS

HORSES

Twenty-two horses, 17 cases with ataxia and 5 control horses, were included in this study. All horses were presented at the faculty of Veterinary Medicine between 2008 and 2017. All were clinically evaluated by a veterinarian with more than 3 years of experience in neurological examinations and afterwards TMS-MMEP, diagnostic imaging and histopathology were performed. The 5 control horses (2 female, 3 male castrated) had a median age of 7 (range 5-19) years and a mean weight of 550 (437-655) kg. None of the control horses showed any abnormality during neurological examination. The group of ataxic horses included 8 intact males, 4 females and 5 castrated males. Their median age was 4 (0.5-20) years, their mean bodyweight 426 (180-572) kg. All ataxic horses had normal consciousness, no cranial nerve deficits and blindfolding did not trigger a head tilt. They showed a mean grade of ataxia of 3.6/5 (range 1-5) (Mayhew, 2008). Except horse number 12, all horses showed ataxia in both thoracic and pelvic limbs, suggesting a cervical spinal cord dysfunction. Horse 12 showed a severe ataxia in the pelvic limbs only. A thoracolumbar lesion was suspected.

TMS WITH MMEP RECORDING

For TMS-MMEP, the procedure described by Rijckaert et al. (2018) was followed. Each horse was sedated with a combination of detomidine (12 µg/kg bodyweight, Domidine, Eurovet Animal Health, Bladel, The Netherlands) and butorphanol (12 µg/kg bodyweight, Dolorex, MSD Animal Health, Boxmeer, The Netherlands). A magnetic stimulator (Magstim 200, The Magstim Company Ltd, Whitland, United Kingdom) and a round 70 mm coil were used to generate a maximal magnetic field of 4 Tesla at the coil surface. The coil was centred over the forehead and maximal stimulus intensity (100%) was applied (Nollet et al., 2004). A standard

electromyograph (Medelec Sapphire, Medelec Ltd, Surrey, United Kingdom) recorded the muscle responses from the tibialis cranialis and the extensor carpi radialis muscle through intramuscular needle (25 mm monopolar, disposable, insulated, stainless steel needle, TECA Corporation, Pleasantville, New York, USA) or adhesive surface electrodes (Skintact FS50, Skintact, Innsbruck, Austria). These electrode types do not have a significant influence on latency time (Rijckaert et al., 2018). One limb at a time was tested, starting at the left pelvic limb, going to the right pelvic, left thoracic and finally right thoracic limb (Rijckaert et al., 2018). For each limb, 4 sequential muscle responses were recorded. For each elicited MMEP, latency time, which is the time interval between the trigger and the first deflection from the baseline, was measured in milliseconds (ms). For each horse, 1 mean latency time was calculated for the thoracic limbs (mean of 8 thoracic measurements, 4 left and 4 right) and 1 mean latency time for the pelvic limbs (mean of 8 pelvic measurements). All MMEP measurements were performed by 1 blinded operator.

DIAGNOSTIC IMAGING

For all horses, lateral radiographs of the cervical vertebrae were made from the occiput to the first thoracic vertebra with a ceiling mounted Phillips X-Ray tube (80 kW). Output parameters varied from 70 kV/25 mAs for the cranial cervical vertebrae to 90 kV/90 mAs for C7-T1. A CR system (Agfa DXM) was used with a grid. All radiographs were anonymized and evaluated for any abnormalities by a blinded, board certified radiologist. Additionally, the intra- and intervertebral sagittal diameter ratios of the vertebral canal were measured at each cervical vertebra as described by Hahn et al. (2008). For both ratios a cut-off value of 0.485 was used to distinguish between a normal and a narrowed vertebral canal resulting in spinal cord compression (Hahn et al., 2008).

If horses showed ataxia on the clinical examination, but standard lateral radiographs did not reveal any abnormalities explaining the clinical signs, additional imaging was performed. This included additional radiographic projections such as oblique and ventrodorsal views, a myelogram or a combination of both. Myelograms were performed under general anaesthesia (triple drip with ketamine, guaiacol glyceryl ether and detomidine or isoflurane inhalation anaesthesia) with injection of iodinated contrast medium (Omnipaque 350, 0,2 mL/kg, diluted

30%) in the subarachnoid space. A similar volume of cerebrospinal fluid was withdrawn from the subarachnoid space prior to injection of the contrast medium. To visualize dynamic spinal cord compression, radiographs were taken with the neck in neutral, flexed and extended position. Compression sites were identified by more than 50% reduction of the dorsal contrast column or more than 20% reduction of the dural diameter (van Biervliet et al., 2004).

HISTOLOGY

All horses were necropsied immediately after euthanasia to minimize post-mortem artefacts. Samples of the cerebrum (3 samples of the rostral, middle and caudal part bilaterally), cerebellum (1 sample, cerebellum-pontine area), brainstem (1 sample, medulla oblongata), cervical (7 samples, C1-C7), thoracic (2 samples, T2-T3 and T7-T8), lumbar (2 samples, L1-L2 and L4-L5) spinal cord and cauda equina (1 sample) were fixed in a phosphate-buffered formaldehyde solution and embedded in paraffin wax. Four micrometre thick sections were stained with haematoxylin and eosin (HE). T-lymphocytes were visualized using a polyclonal rabbit anti-CD3 antibody (Dako, Glostrup, Denmark). All tissues were immune labeled with anti-synaptophysin to show degenerating neurons (Dako, Glostrup, Denmark). A standard avidin–biotin complex method with diaminobenzidine as chromogen was used for visualization (Envision, Dako, Glostrup, Denmark). All sections were assessed blinded by a board certified veterinary pathologist for inflammatory, degenerative or neoplastic changes. The presence of T-lymphocytes in the parenchyma and meninges was evaluated with grade 0 representing no infiltration, grade 1 mild, grade 2 moderate and grade 3 severe infiltration. Also the degree of neuronal and axonal degeneration (spheroids) was scored with grade 0 representing no degeneration, grade 1 mild, grade 2 moderate and grade 3 severe degeneration. T-lymphocytes infiltration or degree of degeneration was called mild if there were locally a few lymphocytes or spheroids visible, moderate if they were visible locally but in large numbers and severe if they were present in large numbers on different sites through the nervous tissue.

STATISTICS

For each series of 8 stimulations, the mean value was calculated and entered as elementary unit to the dataset. To determine possible significant weight and age differences in case and control animals, ANOVA was used. The outcome variable of interest was latency time, which was first checked for a normal distribution.

ANOVA was used to assess the effect of control/ataxic horse on latency time values. A separate model was built for the thoracic and pelvic limbs. To determine the optimal cut-off latency time to differentiate an ataxic horse from a control animal, receiver operating curves were made, separately for thoracic and pelvic limbs. The Youden's index (sensitivity + specificity – 1) was calculated to determine optimal cut-off values. Sensitivity, specificity and their 95% confidence intervals were determined. All analyses were done in IBM SPPS, version 25 and WINEPISCOPE 2.0.

RESULTS

None of the control horses showed abnormalities on the plain radiographs and all inter- and intra-vertebral ratios were above 50%. None of these horses showed significant degenerative or inflammatory changes on histopathology.

In 14/17 horses with clinical signs of ataxia, diagnostic imaging suggested spinal cord compression and spinal cord disease was confirmed by histopathology. Seven horses had indications of spinal cord compression on cervical radiographs. Two of them were suspected to have a neoplastic lesion as they showed severe osteolysis of the vertebral body. The other 5 had at least 1 cervical site with an intra- or intervertebral ratio below 0.485. In the other 7 horses, additional radiographs or myelogram were required for diagnosis as cervical radiographs were normal. In 6 of these horses, the myelogram suggested cervical spinal cord compression. In the other horse, radiographs of the thoracic part of the spine revealed a severe vertebral malformation of T8-T9. The vertebral bodies were shorter compared with surrounding vertebrae, wedge shaped and displaced dorsally. On histopathology, clear degenerative and inflammatory changes were seen. Only the pelvic limb latency times of this horse were included further in the study.

The remaining 3/17 ataxic horses had normal cervical radiographs and myelogram. No radiographs of thoracic or lumbar region were taken. On histopathology, 1 of these horses showed only very mild degenerative and inflammatory lesions, another only showed a moderate mononuclear inflammation and the third had moderate degenerative lesions at the level of the cervical spinal cord.

There was no significant difference in age between both groups, but ataxic horses had a significantly (*P*=0.024) lower weight than control horses. Mean±sd latency time in normal horses was 20±1 ms in thoracic limbs and 39±1 ms in pelvic limbs. Mean±sd latency time values for the ataxic horses were 34±16 ms in the thoracic limbs and 78±26 ms in the pelvic limbs. Latency time values from both thoracic (the thoracic latency time value of the horse with the thoracic lesion was not included) and pelvic limbs were significantly different from those in the control horses (*P*=0.048 and *P*=0.004, respectively). All individual data are presented in table 1.

Figure 1 shows the ROC curves for thoracic and pelvic limb latency times based on 21 and all 22 horses, respectively. The optimal cut-off values for latency time to detect spinal cord dysfunction in ataxic horses were 22 ms (sensitivity (95% CI interval) = 88% (73-100%), specificity (95% CI interval) = 100% (100-100%)) in thoracic and 40 ms (sensitivity=94% (83-100%), specificity=100% (100-100%)) in pelvic limbs. If the 2 ataxic horses without clear compression of the spinal cord (only inflammatory or very mild degenerative lesions), were excluded, the optimal cut-off value for the thoracic limbs remained 22 ms, but the sensitivity and specificity increased to 100 (100-100)% each. The suggested optimal cut-off value for the pelvic limbs, however, was 43 ms with an increase in sensitivity as consequence (sensitivity=100% (100-100%), specificity=100% (100-100%)).

Table 1: Individual results of medical imaging, histopathology and mean latency for control and ataxic horses. (NP=not performed)

Nr.	Sex	Age (years)	Weight (kg)	Grade of ataxia	Minimal vertebral ratio	Reduction dural column (%)	Reduction dorsal contrast column (%)	Degenerative score histology	Inflammatory score histology	Mean latency pelvic limbs (ms)	Mean latency thoracic limbs (ms)	Notes
CONTROL HORSES												
1	mare	13	535	0	> 0.50	NP	NP	1	0	37.5	19.6	
2	gelding	7	655	0	> 0.50	NP	NP	0	0	39.8	20.3	
3	gelding	19	612	0	> 0.50	NP	NP	1	0	39.1	21.1	
4	mare	6	512	0	> 0.50	NP	NP	0	1	39.5	20.1	
5	gelding	5	437	0	> 0.50	NP	NP	0	0	39.0	19.5	
ATAXIC HORSES												
6	gelding	5	515	1	> 0.50	5	12	1	1	39.3	20.0	
7	stallion	4	405	3	0.481	NP	NP	3	2	100.2	24.7	
8	stallion	2	472	3	0.482	54	87	3	1	79.6	25.5	
9	gelding	7	550	3	> 0.50	34	52	3	1	53.5	25.7	
10	mare	3	495	3	> 0.50	27	87	2	1	61.3	22.2	
11	stallion	1	420	3	0.443	20	62	2	1	72.5	29.3	
12	mare	0.5	180	4	> 0.50	NP	NP	2	2	45.8	17.2	Thoracic lesion
13	stallion	2	487	4	0.483	25	64	2	2	87.9	26.2	
14	gelding	18	572	4	> 0.50	16	84	1	2	61.3	38.0	Neoplasia
15	stallion	2	450	4	0.454	NP	NP	3	2	100.0	61.2	
16	mare	19	480	4	> 0.50	25	86	3	0	100.0	38.6	
17	stallion	2	375	4	> 0.50	21	77	3	2	100.0	22.2	
18	gelding	4	450	4	> 0.50	9	21	1	2	40.5	23.3	
19	stallion	4	361	4	> 0.50	12	37	2	1	121.0	56.5	
20	gelding	20	470	4	> 0.50	NP	NP	3	2	55.3	28.3	Neoplasia
21	stallion	16	292	5	> 0.50	32	100	3	1	100.0	65.0	
22	mare	20	275	5	> 0.50	29	74	2	1	100.0	61.4	Neoplasia

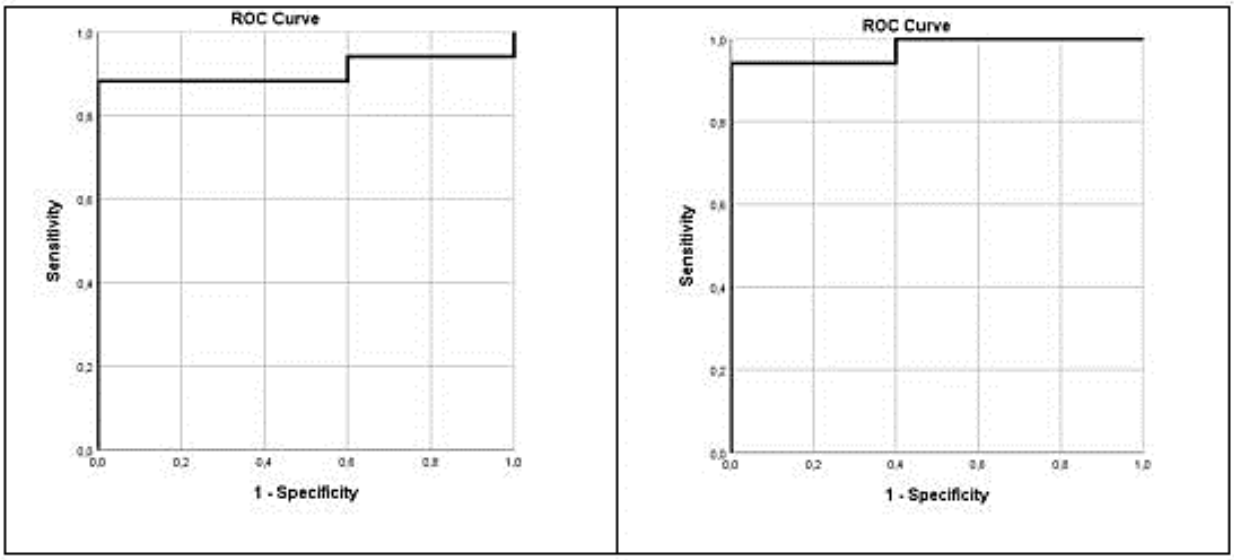


Figure 1. ROC curves for thoracic (left) and pelvic (right) limb latencies based on 22 horses.

DISCUSSION

The purpose of this study was to determine cut-off values of MMEP latency time for spinal cord dysfunction in horses, confirmed by narrowing of the vertebral canal on diagnostic imaging and inflammatory or degenerative lesions on histopathology. There were a number of limitations. First, the weight of the control horses differed significantly from the ataxic horses. Nollet et al. (2004) found a significant influence of weight on latency time in the univariate analysis. In the multivariable analysis weight was excluded because of the strong correlation with wither height. But, the weight difference in the present study could have an influence on MMEP results. However, as the weight of the ataxic horses is lower, they are probably smaller, and a shorter latency time would be expected compared to the heavier, and probably taller, control horses.

Secondly, the present calculations were made on only 5 control horses. Five control animals is not a lot, but the differences between both groups were already significant. Furthermore, the variation in latency time between the different control animals is small, as we expected from former MMEP research in larger numbers of healthy horses (Nollet et al., 2004; Rijckaert et al., 2018). Adding control animals will only decrease variation even more and make the difference between control horses and cases more clear. However, this small advantage does

not outweigh the ethical considerations about adding extra healthy horses. So, the number of controls was kept as low as possible. The fact that all control horses were adult while in the ataxic group also very young horses were included neither should be an issue, as age does not have a significant influence on latency time in horses (Nollet et al., 2004). A more important problem is that the ataxic horses had a high grade of ataxia (mean 3.6/5). In these obviously ataxic horses, TMS might be unnecessary. TMS is especially useful in the subtle cases, where additional confirmation of neurological dysfunction is wanted. In the present study however, only 1 horse with a grade 1 and no horses with a grade 2 were included. Therefore, the present cut-off values need to be validated in future TMS studies on larger numbers of horses, including subtle ataxia cases.

Third, not all horses, and none of the control horses, were examined by myelography. In the ataxic horses without myelogram, cervical radiographs already indicated spinal cord compression which was confirmed by histopathology. In the control horses, it is unlikely that a myelogram would have shown spinal cord compression, as histopathology was normal.

Fourth, only lateral cervical radiographs were made and no attention was paid to enlargement of the caudal articular process joints. These enlarged joints do not necessarily result in spinal cord compression (Down and Henson, 2009) but in some cases they will.

Lastly, conclusions were made based on histopathology, which is not a perfect gold standard. Functional deficits can occur before histopathological changes can be found and influence of age and post-mortem artefacts can impede interpretation. However, it is currently the most accurate and sensitive test available (Mitchell et al., 2012; Van Biervliet, 2007).

These limitations in mind, the present study defines clear cut-off values for thoracic and pelvic limb MMEP latency times, with a high sensitivity and specificity to diagnose spinal cord dysfunction in horses. The results are in line with a comparable study in Doberman Pinscher dogs where latency time cut-off values were calculated to discriminate between normal and affected animals, based on the findings of clinical examinations and magnetic resonance imaging (MRI). Comparable to our results, in these dogs, the pelvic limb latency times had the highest sensitivity and overall MMEP sensitivities and specificities were very high (about 90%)(De Decker et al., 2011).

Similar to the horses in the present study, significantly different latency times were found in dogs with and without cervical spinal cord disease (Amendt et al., 2017; da Costa et al., 2006; Martin-Vaquero and da Costa, 2014; Poma et al., 2002). In these studies MMEP results were always compared with MRI findings but there was no histopathological confirmation of spinal cord disease. MRI is a popular, non-invasive and very sensitive technique, although not all lesions on MRI cause functional deficits. In horses, MRI scanning facilities are not always available and only post-mortem cervical MRI studies have been published (Janes et al., 2014; Mitchell et al., 2012; Sleutjens et al., 2014). MRI examinations seem to be more accurate than cervical radiographs to diagnose CVM (Janes et al., 2014) but sensitivity and positive predictive values remain poor to moderate, compared to histopathological diagnosis (Mitchell et al., 2012).

There was a good agreement between clinical examination, diagnostic imaging and histopathology. Only in 3/22 horses, there were contradictory results. Three ataxic cases were evaluated as normal on diagnostic imaging. This might be because there was no compression or because the compression was not diagnosed. False negative results are possible in cases of lateral compression, enlarged articular process joints or because of a the low sensitivity of the used cut-off values. For the plain radiographs 0.485 was used as cut-off value for cervical vertebral sagittal ratios, but also 0.52 and 0.56 have been proposed in earlier research (Moore et al., 1994). Logically, sensitivity to identify spinal cord compression will be higher using 0.52 and 0.56, but also the rate of false positives will increase. The same problem exists for myelography. Several cut-off values have been proposed, but it remains difficult to combine acceptable sensitivity and specificity in 1 number (van Biervliet et al., 2004).

On histopathology the difference between controls and ataxic horses was obvious, in general: controls had only very mild degenerative or inflammatory lesions, possibly a consequence of age, while most ataxic horses showed mild to moderate inflammatory changes and moderate to severe degenerative changes. Two ataxic horses did not have clear pathologic signs of spinal cord compression. In these 2 horses, latency time values were only slightly increased. Horse 6 even had mean latency time values below the cut-off values. In combination with only a grade 1 ataxia, the question arises whether the horse did have a neurological problem or whether an orthopaedic cause would be more likely. Nollet et al. (2005) suggested that TMS could help in differentiating between orthopaedic and neurological causes of recumbency such as severe

laminitis or physitis, but no data are available about more subtle cases. As no cases with clear orthopaedic gait deficits were included in the study, no further conclusions can be made about this hypothesis. The other horse (number 18) without clear signs of spinal cord compression, had a thoracic latency time slightly above the threshold and moderate signs of inflammation in the spinal cord in combination with a grade 4 ataxia. Possibly, the ataxia was caused by another etiology than spinal cord compression (e.g. EPM, EDM/NAD or equine herpesvirus myeloencephalopathy). The present study confirms the test can detect spinal cord compression, but in the future, it might be interesting to evaluate the sensitivity in cases with an etiology of ataxia other than spinal cord compression too.

In conclusion, results of the present study suggest 22 ms for thoracic and 43 ms for pelvic limbs as optimal cut-off values to differentiate spinal cord dysfunction caused by spinal cord compression in horses with a high grade ataxia, from neurologically healthy animals. Using these threshold values for latency time, a much higher sensitivity and specificity than currently reported for cervical radiography was obtained. These results are promising and merit validation of these cut-offs on a larger dataset, including animals with mild ataxia and gait deficits of orthopaedic nature in future work.

REFERENCES

Amendt, H.L., Siedenburg, J.S., Steffensen, N., Kordass, U., Rohn, K., Tipold, A., Stein, V.M., 2017. Correlation between severity of clinical signs and transcranial magnetic motor evoked potentials in dogs with intervertebral disc herniation. Veterinary Journal 221, 48-53.

da Costa, R.C., Poma, R., Parent, J.M., Partlow, G., Monteith, G., 2006. Correlation of motor evoked potentials with magnetic resonance imaging and neurologic findings in Doberman Pinschers with and without signs of cervical spondylomyelopathy. American Journal of Veterinary Research 67, 1613-1620.

De Decker, S., Van Soens, I., Duchateau, L., Gielen, I.M.V.L., van Bree, H.J.J., Binst, D.H.A.R., Waelbers, T., Van Ham, L.M.L.M., 2011. Transcranial magnetic stimulation in Doberman Pinschers with clinically relevant and clinically irrelevant spinal cord compression on magnetic resonance imaging. Journal of the American Veterinary Medical Association 238, 81-88.

Down, S.S., Henson, F.M., 2009. Radiographic retrospective study of the caudal cervical articular process joints in the horse. Equine Veterinary Journal 41, 518-524.

Hahn, C.N., Handel, I., Green, S.L., Bronsvoort, M.B., Mayhew, I.G., 2008. Assessment of the utility of using intra- and intervertebral minimum sagittal diameter ratios in the diagnosis of cervical vertebral malformation in horses. Veterinary Radiology and Ultrasound 49, 1-6.

Hamir, A.N., Moser, G., Rupprecht, C.E., 1992. A five year (1985-1989) retrospective study of equine neurological diseases with special reference to rabies. Journal of comparative pathology 106, 411-421.

Hughes, K.J., Laidlaw, E.H., Reed, S.M., Keen, J., Abbott, J.B., Trevail, T., Hammond, G., Parkin, T.D., Love, S., 2014. Repeatability and intra- and inter-observer agreement of cervical vertebral sagittal diameter ratios in horses with neurological disease. Journal of Veterinary Internal Medicine 28, 1860-1870.

Janes, J.G., Garrett, K.S., McQuerry, K.J., Pease, A.P., Williams, N.M., Reed, S.M., MacLeod, J.N., 2014. Comparison of magnetic resonance imaging with standing cervical radiographs for evaluation of vertebral canal stenosis in equine cervical stenotic myelopathy. Equine Veterinary Journal 46, 681-686.

Levine, J.M., Adam, E., MacKay, R.J., Walker, M.A., Frederick, J.D., Cohen, N.D., 2007. Confirmed and presumptive cervical vertebral compressive myelopathy in older horses: A retrospective study (1992-2004). Journal of Veterinary Internal Medicine 21, 812-819.

Levine, J.M., Ngheim, P.P., Levine, G.J., Cohen, N.D., 2008. Associations of sex, breed, and age with cervical vertebral compressive myelopathy in horses: 811 cases (1974-2007). Journal of the American Veterinary Medical Association 233, 1453-1458.

Levine, J.M., Scrivani, P.V., Divers, T.J., Furr, M., Mayhew, I.J., Reed, S., Levine, G.J., Foreman, J.H., Boudreau, C., Credille, B.C., Tennent-Brown, B., Cohen, N.D., 2010. Multicenter case-control study of signalment, diagnostic features, and outcome associated with cervical vertebral malformation-malarticulation in horses. Journal of the American Veterinary Medical Association 237, 812-822.

Martin-Vaquero, P., da Costa, R.C., 2014. Transcranial magnetic motor evoked potentials in Great Danes with and without clinical signs of cervical spondylomyelopathy: Association with neurological findings and magnetic resonance imaging. Veterinary Journal 201, 327-332.

Mayhew, I.G., 2008. Large Animal Neurology - neurological evaluation form, 2nd ed. Wiley-Blackwell, Chichester.

Mitchell, C.W., Nykamp, S.G., Foster, R., Cruz, R., Montieth, G., 2012. The use of magnetic resonance imaging in evaluating horses with spinal ataxia. Veterinary Radiology and Ultrasound 53, 613-620.

Moore, B.R., Reed, S.M., Biller, D.S., Kohn, C.W., Weisbrode, S.E., 1994. Assessment of vertebral canal diameter and bony malformations of the cervical part of the spine in horses with cervical stenotic myelopathy. American Journal of Veterinary Research 55, 5-13.

Nappert, G., Vrins, A., Breton, L., Beauregard, M., 1989. A Retrospective Study of 19 Ataxic Horses. Canadian Veterinary Journal -Revue Veterinaire Canadienne 30, 802-806.

Nollet, H., Deprez, P., Van Ham, L., Verschooten, F., Vanderstraeten, G., 2002. The use of magnetic motor evoked potentials in horses with cervical spinal cord disease. Equine Veterinary Journal 34, 156-163.

Nollet, H., Deprez, R., Van Ham, L., Dewulf, J., Decleir, A., Vanderstraeten, G., 2004. Transcranial magnetic stimulation: normal values of magnetic motor evoked potentials in 84 normal horses and influence of height, weight, age and sex. Equine Veterinary Journal 36, 51-57.

Nollet, H., Van Ham, L., Dewulf, J., Vanderstraeten, G., Deprez, P., 2003a. Standardization of transcranial magnetic stimulation in the horse. Veterinary Journal 166, 244-250.

Nollet, H., Van Ham, L., Gasthuys, F., Dewulf, J., Vanderstraeten, G., Deprez, P., 2003b. Influence of detomidine and buprenorphine on motor-evoked potentials in horses. Veterinary Record 152, 534-537.

Nollet, H., Van Ham, L., Verschooten, F., Vanderstraeten, G., Deprez, P., 2003c. Use of magnetic motor-evoked potentials in horses with bilateral hind limb ataxia. American Journal of Veterinary Research 64, 1382-1386.

Nollet, H., Vanschandevijl, K., Van Ham, L., Vanderstraeten, G., Deprez, P., 2005. Role of transcranial magnetic stimulation in differentiating motor nervous tract disorders from other causes of recumbency in four horses and one donkey. Veterinary Record 157, 656-658.

Nout, Y.S., Reed, S.M., 2003. Cervical vertebral stenotic myelopathy. Equine Veterinary Education 15, 212-223.

Oswald, J., Love, S., Parkin, T.D., Hughes, K.J., 2010. Prevalence of cervical vertebral stenotic myelopathy in a population of thoroughbred horses. Veterinary Record 166, 82-83.

Poma, R., Parent, J.M., Holmberg, D.L., Partlow, G.D., Monteith, G., Sylvestre, A.M., 2002. Correlation between severity of clinical signs and motor evoked potentials after transcranial magnetic stimulation in large-breed dogs with cervical spinal cord disease. Journal of the American Veterinary Medical Association 221, 60-64.

Rijckaert, J., Pardon, B., Van Ham, L., van Loon, G., Deprez, P., 2018. Magnetic Motor Evoked Potential Recording in Horses Using Intramuscular Needle Electrodes and Surface Electrodes. Journal of Equine Veterinary Science 68, 101-107.

Sleutjens, J., Cooley, A.J., Sampson, S.N., Wijnberg, I.D., Back, W., van der Kolk, J.H., Swiderski, C.E., 2014. The equine cervical spine: comparing MRI and contrast-enhanced CT images with anatomic slices in the sagittal, dorsal, and transverse plane. The Veterinary quarterly 34, 74-84.

Szklarz, M., Skalec, A., Kirstein, K., Janeczek, M., Kasparek, M., Kasparek, A., Waselau, M., 2018. Management of equine ataxia caused by cervical vertebral stenotic myelopathy: A European perspective 2010-2015. Equine Veterinary Education 30, 370-376.

Tyler, C.M., Davis, R.E., Begg, A.P., Hutchins, D.R., Hodgson, D.R., 1993. A Survey of Neurological Diseases in Horses. Australian Veterinary Journal 70, 445-449.

Van Biervliet, J., 2007. An evidence-based approach to clinical questions in the practice of equine neurology. The Veterinary clinics of North America. Equine practice 23, 317-328.

van Biervliet, J., Scrivani, P.V., Divers, T.J., Erb, H.N., de Lahunta, A., Nixon, A., 2004. Evaluation of decision criteria for detection of spinal cord compression based on cervical myelography in horses: 38 cases (1981-2001). Equine Veterinary Journal 36, 14-20.

Walmsley, J.R., 2005. Surgical treatment of cervical spinal cord compression in horses: a European experience. Equine Veterinary Education 17, 39-43.

CHAPTER 7

ACCURACY OF TRANSCRANIAL MAGNETIC STIMULATION FOR DIAGNOSIS OF SPINAL CORD DYSFUNCTION IN HORSES

ACCURACY OF TRANSCRANIAL MAGNETIC STIMULATION FOR DIAGNOSIS OF SPINAL CORD DYSFUNCTION IN HORSES

J. Rijckaert[1]., Raes E[2]., Buczinski S[3]., Van Ham L.[4], Deprez, P[1], van Loon G.[1], Pardon B.[1]

[1] Department of Large Animal Internal Medicine, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, Merelbeke 9820, Belgium

[2] Department of Veterinary Medical Imaging and Small Animal Orthopedics, Ghent University, Salisburylaan 133, Merelbeke 9820, Belgium

[3] Clinical Science Department, Faculty of Veterinary Medicine, University of Montreal, 200 Rue Sicotte, Saint-Hyacinthe, QC J2S 2M2, Canada

[4] Small Animal Department, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, Merelbeke 9820, Belgium

Adapted from: Rijckaert, J., Raes, E., Buczinski, S., Van Ham, L., Deprez, P., van Loon, G., Pardon, B. Accuracy of transcranial magnetic stimulation for diagnosis of spinal cord dysfunction in horses. Journal of Veterinary Internal Medicine (under review).

ABSTRACT

Spinal cord dysfunction and ataxia are common in horses. Presumptive diagnosis is most commonly based on neurological examination and cervical radiography, but the interest into the diagnostic value of transcranial magnetic stimulation (TMS) with recording of magnetic motor evoked potentials has increased. Problematic for the evaluation of diagnostic tests for spinal cord dysfunction is the absence of a gold standard in the living animal. Therefore the objective of the present study was to compare the diagnostic accuracy of TMS, cervical radiography and clinical examination using a Bayesian framework to account for the absence of a gold standard. For TMS, the optimal diagnostic criterion for spinal cord dysfunction diagnosis was determined. This study was done on 174 horses admitted at the clinic for neurological examination. Bayesian estimate of the prevalence (95% CI) of spinal cord dysfunction was 58.1 (48.3-68.3)%. For TMS, highest accuracy was obtained using the minimum latency time for the pelvic limbs (Youden's index = 0.85). In all evaluated models, cervical radiography performed poorest. Sensitivity and specificity of neurological examination were 97.6 (91.4-99.9)% and 74.7 (61.0-96.3)%, for radiography they were 43.0 (32.3-54.6)% and 77.3 (67.1-86.1)%, respectively. TMS reached a sensitivity and specificity of 87.5 (68.2-99.2)% and 97.4 (90.4-99.9)%. In conclusion, TMS-MMEP was the best test to diagnose spinal cord disease, the neurological examination was second best, but the accuracy of cervical radiography was low. Selecting animals based on neurological examination (highest sensitivity) and confirming disease by TMS-MMEP (highest specificity) would be the best diagnostic strategy.

Key words: magnetic motor evoked potentials, cervical radiographs, ataxia, cervical vertebral malformation, myelogram

INTRODUCTION

Spinal cord dysfunction and ataxia are common in horses. In the USA, Equine protozoal myeloencephalitis is an important cause, but worldwide, cervical vertebral compressive myelopathy (CVCM) and neuroaxonal dystrophy (NAD)/equine degenerative myeloencephalopathy (EDM) are the most important etiologies (Burns and Finno, 2018; Tyler et al., 1993). On NAD/EDM affected farms, prevalence can be high (Finno et al., 2013) and might even raise up to about 60% (Aleman et al., 2011). For CVCM, several studies reported hundreds of cases (Levine et al., 2008; Levine et al., 2010; Papageorges et al., 1987). Because ataxic horses are no longer suitable for riding purposes and a suspected genetic background makes them less favourable for breeding, a positive diagnosis often leads to euthanasia.

Given these huge consequences of a positive diagnosis, the absence of a true gold standard test for CVCM or EDM/NAD in living horses is problematic. EDM affected horses often have a low serum vitamin E level (Aleman et al., 2011), but for definite diagnosis histopathology is required. CVCM can be detected by myelography, computed tomography (CT) and cervical radiography, but all these techniques still have limitations. CT scans, large enough to visualize C7 are expensive, still poorly available and do not enable flexion and extension of the neck to evaluate dynamic compression of the spinal cord. With myelography spinal cord compression can be visualized with the neck in flexed and extended position, but general anaesthesia is required and the sensitivity appears rather low (van Biervliet et al., 2004), especially for the cranial parts of the neck. Cervical radiography might indicate narrowing of the vertebral canal, but accuracy is actually too low for definite diagnosis (Janes et al., 2014; Levine et al., 2007; Levine et al., 2010; Papageorges et al., 1987). So, a presumptive diagnosis is often based on the history of the patient and the clinical neurological examination. However, certainly in subtle cases, the agreement between observers performing a neurological examination is poor and differentiation from orthopaedic causes may be challenging (Olsen et al., 2014; Saville et al., 2017).

Transcranial magnetic stimulation (TMS) with recording of magnetic motor evoked potentials (MMEPs) is a promising additional test for diagnosis of spinal cord dysfunctions in horses (Nollet et al., 2002; Nollet et al., 2004; Nollet et al., 2003a; Nollet et al., 2003b; Nollet et al., 2005; Rijckaert et al., 2018). A magnetic 70mm coil is placed on the head of the horse, at the

level of the brain, to perform a magnetic stimulation. This induces descending volleys through the spinal cord, evoking a muscle contraction reflected by the MMEP on the EMG machine. On each MMEP, several parameters can be measured, but the latency time, the time between stimulation and onset of muscle contraction, is most reliable (Nollet et al., 2004; Rijckaert et al., 2018). In these studies, the mean latency time of 4 MMEPs is used for analysis. In human medicine, the minimal latency time is used instead of the mean, but in horses these have not been evaluated yet. In normal horses, mean latency time is short and has a low standard deviation while in horses with spinal cord disease, latency time is more variable and clearly prolonged (Nollet et al., 2002; Nollet et al., 2003b; Nollet et al., 2005). A recent study that compared TMS with histopathology showed that for diagnosis of spinal cord dysfunction, the optimal cut-off values for latency time were 22 ms in the thoracic and 43 ms in the pelvic limbs (Rijckaert et al., JVIM accepted june 2019). However, these values have not been validated yet and neurological examination and cervical radiography have not been evaluated in a Bayesian framework either. Therefore the objectives of the present study were to compare the diagnostic accuracy of TMS, cervical radiography and clinical examination using a Bayesian framework to account for the absence of a gold standard and to determine the optimal diagnostic criterion for spinal cord dysfunction diagnosis by TMS.

MATERIAL AND METHODS

STUDY PROTOCOL AND HORSES

A diagnostic, retrospective test study was performed. The study population consisted of 174 (99 male castrated, 28 intact male and 47 female) horses. All horses were presented between 2008 and 2018 at Ghent University clinic for confirmation or exclusion of a neurological gait abnormality. Al horses were evaluated by a neurological examination, transcranial magnetic stimulation and cervical radiography. On 75 horses, an orthopedic examination was performed as orthopedic disease was suspected, but the results were not included in the study.

EXAMINATION

Neurological examination

Each horses neurological function was examined by at least 1 of 5 veterinarians. All examiners had at least 3 years of experience in performing neurological examinations. A standard protocol was followed, evaluating the consciousness of the horses, posture and stance, cranial nerves and muscle atrophy in stance and hypermetric movements, weakness, irregular irregularities during walk, trot, canter and transitions and during circling, backing, tail pull and blindfolding (Mayhew, 2008). If no abnormalities were seen, the horses had a grade 0 of ataxia, if only subtle our doubtful lesions were seen, the horses received a grade 1. If there were mild abnormalities seen, a grade 2 was given, in case of clear incoordination at all times but no falling, a grade 3. Horses who fell or had a tendency to do so, received a grade 4 and if they were recumbent they were grade 5 ataxic (Mayhew, 1978).

Cervical radiography

For all horses, lateral radiographs of the cervical vertebrae were made from the occiput to the first thoracic vertebra with a ceiling mounted Phillips X-Ray tube (80 kW). Output parameters varied from 70 kV/25 mAs for the cranial cervical vertebrae to 90 kV/90 mAs for C7-T1. A CR system (Agfa DXM) was used with a grid. All radiographs were anonymized and evaluated for any abnormalities by a blinded, board certified radiologist. Additionally, the intra- and intervertebral sagittal diameter ratios of the vertebral canal were measured at each cervical vertebra as described by Hahn et al. (2008). For both ratios a cut-off value of 0.485 was used to distinguish between a normal and a narrowed vertebral canal indicative for spinal cord compression (Hahn et al., 2008).

Transcranial magnetic stimulation

For TMS-MMEP, the procedure described by Nollet et al. (2004) was followed. Each horse was sedated with a combination of detomidine (12 µg/kg bodyweight, Domidine, Eurovet Animal Health, Bladel, The Netherlands) and butorphanol (12 µg/kg bodyweight, Dolorex, MSD Animal Health, Boxmeer, The Netherlands). A magnetic stimulator (Magstim 200, The Magstim Company Ltd, Whitland, United Kingdom) and a round 70 mm coil were used to generate a maximal magnetic field of 4 Tesla at the coil surface. The coil was centred over the forehead and maximal stimulus intensity (100%) was applied (Nollet et al., 2004). A standard

electromyograph (Medelec Sapphire, Medelec Ltd, Surrey, United Kingdom) recorded the muscle responses from the tibialis cranialis and the extensor carpi radialis muscle through intramuscular needle (25 mm monopolar, disposable, insulated, stainless steel needle, TECA Corporation, Pleasantville, New York, USA) or adhesive surface electrodes (Skintact FS50, Skintact, Innsbruck, Austria). These electrode types do not have a significant influence on latency time (Rijckaert et al., 2018). One limb at a time was tested, starting at the left pelvic limb, going to the right pelvic, left thoracic and finally right thoracic limb (Rijckaert et al., 2018). For each limb, 4 sequential muscle responses were recorded. For each elicited MMEP, latency time, which is the time interval between the trigger and the first deflection from the baseline, was measured in milliseconds (ms). The cut-off values used for MMEP onset latency time were 21.7 and 42.8 ms for thoracic and pelvic limbs, respectively, based on former performed histopathological research (Rijckaert et al., JVIM accepted june 2019). If latency time was shorter, TMS-MMEP was negative indicating a normal motor conduction through the spinal cord. If latency time was equal or longer than the cut-off, TMS-MMEP was positive indicating an abnormal motor function. All latency time measurements were performed by 1 blinded operator.

STATISTICS

Definition of outcome tested

As there is currently no gold standard in horses to detect spinal cord disease, Bayesian latent class models were used for accuracy calculation. Bayesian latent class models create their own probabilistic definition of the outcome studied, depending on what the tests actually detect (e.g. conductivity or compression by bony structures). In this study, TMS detects conductivity of the spinal cord. Neurologic examination detects ataxia, which is a clinical expression of a sensory disturbance. Cervical radiography visualizes (and measures) the degree of compression of the spinal cord by bony structures. Hence, the latent variable under consideration is best defined as spinal cord dysfunction, as it is the common factor for all three tests.

Model development

In order to assess the accuracy of the three tests, we considered a latent class model allowing for conditional dependence between two tests, namely TMS and radiography. We opted for this model because TMS measures the conductivity of the spinal cord, which is disturbed when compressed by bony structures, as measured by radiology. We modeled conditional dependence as previously described (Dendukuri and Joseph, 2001; Buczinsky et al, 2015). The unknown parameters of interest are the sensitivity and specificity of the three diagnostic tests and the prevalence of spinal cord dysfunction in the study population. A literature search delivered acceptable prior information for sensitivity and specificity of cervical radiography. For prevalence estimation of spinal cord dysfunction, ataxia and TMS, information was limited to best guesses by the authors. For TMS the only available information on diagnostic accuracy was the dataset we previously used to identify optimum cut-off values. Hence, we opted to only use prior information on prevalence and sensitivity/specificity of cervical radiography. In all models we used non-informative priors for Se_{TMS}, Sp_{TMS}, Se_{NeurEx} and $Sp_{aNeurEx}$ (Beta (1,1)). We tested different TMS parameters in this Bayesian framework, comparing with ataxia and radiography. Assessment of model sensitivity to priors was done by evaluating three models. The first model used non-informative priors on prevalence and the three tests. In model 2, prior-information on prevalence of spinal cord dysfunction was added and in model 3 prior information on both prevalence as sensitivity and specificity of radiography were added.

Prior distribution determination process.

Prior information was derived from available literature and expert opinion. As in the present study population including a lot of horses suspected of a neurological disease, the prevalence of spinal cord disease was estimated at 60% with 95% certainty it would be less than 90% (beta (1.4, 3.1)). The range in which the researchers were 95% confident that the true value of the prevalence was above (or below) was obtained from two experts, blinded to each other's guesses. Sensitivity and specificity to detect spinal cord compression on cervical radiography was estimated at 0.50 and 0.70 (Levine et al., 2010) with 95% certainty it would be more than 0.10 and 0.40, respectively. These values were used to determine the beta distribution parameters of the corresponding prior distribution using a free online beta distribution calculator (epitools, Sergeant, ESG, 2013, AusVet animal Health Services and Australian

Biosecurity cooperative Research Centre for Emerging Infectious Diseases) available at http://epitools.ausvet.com.au.

Posterior estimates for each parameter were determined based on a sample from the posterior distribution using Gibbs sampling with the Winbugs statistical freeware (version 1.4.3., MRC Biostatistics unit, Cambridge, UK). Each model was assessed after a burn in of 5000 iterations and a total number of 100,000 iterations. The posterior median and 2.5-97.5 credibility intervals (95% CI) were extracted for each parameters. A total of three chains with different initial values were used. Model convergence was checked by visual inspection of density and Gelman-Rubin plots (Nzoufras, 2009). Plots of chain autocorrelation were inspected to investigate the need of thinning of the chains.

To determine which TMS criterion is most suitable for spinal cord dysfunction we evaluated the following TMS criteria in the Bayesian framework, using the three models described above each time. The criteria evaluated were: mean latency time of 8 thoracic measurements, mean latency time of 8 pelvic limb measurements, minimal latency time of 8 thoracic measurements, minimal latency time of 8 pelvic limb measurements, minimal of 8 thoracic or minimal of 8 pelvic latency times abnormal or minimal of 8 thoracic and minimum of 8 pelvic latency times abnormal. Each time sensitivity and specificity was determined and to identify the TMS criteria with highest combined sensitivity and specificity, the Youden's index was used.

RESULTS

The age of the horses ranged from 1-21 (median 5.5) years and their weight from 230-750 (median 555) kg. Most horses (146) were European warmbloods, 9 were coldblooded types, 4 were quarter horses, 3 Standardbred and 1 was thoroughbred. Eleven horses were presented for pre purchase examination, 58 were suspected to be ataxic, 34 horses showed signs of weakness, 52 presented an atypical lameness and 19 performed poor or were reluctant to work.

All latent class models converged. A conditional dependence scenario was used due to the fact that the study was underpowered to reject conditional dependence. All parameters were relatively stable across the different models with less than 5% variation compared to the posterior medians.

Estimated prevalence of spinal cord dysfunction averaged varied for the different TMS decision criteria between 43.1 (29.3-58.3)% and 60.5 (49.5-70.8)%. For every decision criterion, the variation between the different models was limited to maximal 5%. In table 1, the Youden's indexes for all ran models are shown. The overall best performing test (Youden's index = 0.85) was TMS-MMEP using the minimum latency time for the pelvic limbs. Also for 3 other different decision criteria, TMS-MMEP had the highest Youden's index, indicating it was the best performing diagnostic test. The neurological examination followed on the second place with a maximal index of 0.80. For 5 out of 6 decision criteria, cervical radiography was the poorest test (Youden's index = 0.18-0.31). The highest sensitivity values were found for the neurological examination (0.73-0.99), the highest specificity values were found for TMS-MMEP (0.67-0.97).

The two most valuable TMS-MMEP decision criteria for practice are the minimal latency time of the pelvic limbs (Table 2) and the mean latency time of the pelvic limbs (Table 4). The highest TMS-MMEP sensitivity is achieved by using the mean pelvic limb latency time (sensitivity=0.95) or the minimal latency time of thoracic or pelvic (sensitivity=0.92). The highest TMS-MMEP specificity is achieved using the minimal (specificity=0.97) or mean (specificity=0.86) pelvic latency time.

Table 1. Youden's index for TMS-MMEP, neurological examination and cervical radiography, derived from the informed model 3 of for each TMS-MMEP latency time decision criterion.

		TMS-MMEP	Neurological examination	Cervical radiography
1	**Minimum pelvic**	**0.85**	0.72	0.20
2	**Mean pelvic**	**0.81**	0.80	0.18
3	**Minimum thoracic OR pelvic**	**0.77**	0.63	0.24
4	**Minimum thoracic**	**0.71**	0.49	0.27
5	**Mean thoracic**	0.61	**0.80**	0.31
6	**Minimum thoracic AND pelvic**	0.27	**0.72**	0.62

Table 2. Results of Bayesian latent class modelling using the minimum latency time of the pelvic limbs. (Se_{NeurEx}=sensitivity of neurological examination, Sp_{NeurEx}=specificity of neurological examination, Se_{RX}=sensitivity of cervical radiographs, Sp_{NeurRX}=specificity of cervical radiographs, Se_{MMEP}=sensitivity of TMS-MMEP, Sp_{NeurRX}=specificity of TMS-MMEP, covDp= Covariance for positives , covDn= Covariance for negatives)

	Model 1		Model 2		Model 3	
	Prior densities	Posterior densities	Prior densities	Posterior densities	Prior densities	Posterior densities
Se_{NeurEx}	Beta (1,1)	97.6 (91.1-99.9)	Beta (1,1)	97.6 (91.4-99.9)	Beta (1,1)	97.6 (91.4-99.9)
Sp_{NeurEx}	Beta (1,1)	76.0 (61.6-97.5)	Beta (1,1)	84.8 (61.0-96.1)	Beta (1,1)	74.7 (61.0-96.3)
Se_{RX}	Beta (1,1)	42.6 (31.5-54.6)	Beta (1,1)	42.7 (31.6-54.9)	Beta (3.3,3.3)	43.0 (32.3-54.6)
Sp_{RX}	Beta (1,1)	78.3 (67.4-87.5)	Beta (1,1)	78.1 (67.2-87.3)	Beta (6.3,3.3)	77.3 (67.1-86.1)
Se_{MMEP}	Beta (1,1)	85.9 (67.2-98.7)	Beta (1,1)	87.3 (68.4-99.0)	Beta (1,1)	87.5 (68.2-99.2)
Sp_{MMEP}	Beta (1,1)	97.4 (90.6-99.9)	Beta (1,1)	97.3 (90.4-99.9)	Beta (1,1)	97.4 (90.4-99.9)
Prevalence	Beta (1,1)	49.8 (38.6-63.8)	Beta (1.4, 3.1)	48.4 (37.6-61.9)	Beta (1.4, 3.1)	48.3 (37.8-62.1)
covDp	U (0, a)	-0.0 (-0.06-0.04)	U (0, a)	-0.0 (-0.06-0.04)	U (0, a)	-0.01 (-0.06-0.04)
covDn	U (0, b)	0.0 (-0.01-0.03)	U (0, b)	0.0 (-0.01-0.03)	U (0, b)	0.010 (-0.02-0.03)
Model 1: no informative priors Model 2: informative prior on prevalence of cervical conductive disturbance (mode 60%; 5th percentile = 10%) Model 3: informative priors on prevalence and Se_{RX} (mode 50%; 5th percentile = 10%) and Sp_{RX} (mode 70%; 5th percentile = 40%)						

Table 3. Results of Bayesian latent class modelling using the mean latency time of the pelvic limbs. (Se_{NeurEx}=sensitivity of neurological examination, Sp_{NeurEx}=specificity of neurological examination, Se_{RX}=sensitivity of cervical radiographs, Sp_{NeurRX}=specificity of cervical radiographs, Se_{MMEP}=sensitivity of TMS-MMEP, Sp_{NeurRX}=specificity of TMS-MMEP, covDp= Covariance for positives , covDn= Covariance for negatives)

	Model 1		Model 2		Model 3	
	Prior densities	Posterior densities	Prior densities	Posterior densities	Prior densities	Posterior densities
Se_{NeurEx}	Beta (1,1)	98.3 (91.0-99.9)	Beta (1,1)	98.4 (91.6-99.9)	Beta (1,1)	98.5 (92.2-99.9)
Sp_{NeurEx}	Beta (1,1)	82.3 (67.5-98.4)	Beta (1,1)	81.4 (67.1-97.6)	Beta (1,1)	81.5 (67.2-97.6)
Se_{RX}	Beta (1,1)	40.9 (30.6-51.8)	Beta (1,1)	40.9 (30.7-51.7)	Beta (3.3,3.3)	41.2 (31.0-51.7)
Sp_{RX}	Beta (1,1)	77.8(65.1-89.0)	Beta (1,1)	77.2 (64.4-88.5)	Beta (6.3,3.3)	76.3 (64.6-86.3)
Se_{MMEP}	Beta (1,1)	94.2 (82.6-99.7)	Beta (1,1)	94.7 (83.5-99.7)	Beta (1,1)	94.6 (83.2-99.7)
Sp_{MMEP}	Beta (1,1)	87.3 (75.8-97.2)	Beta (1,1)	86.8 (75.0-96.6)	Beta (1,1)	86.3 (75.3-95.1)
Prevalence.	Beta (1,1)	59.5 (49.4-69.9)	Beta (1.4, 3.1)	58.1 (48.3-68.5)	Beta (1.4, 3.1)	58.1 (48.3-68.3)
covDp	U (0, a)	0.0 (-0.03-0.04)	U (0, a)	0.08 (0.02-0.15)	U (0, a)	0.0 (-0.03-0.04)
covDn	U (0, b)	0.08 (0.01-0.15)	U (0, b)	0.0 (-0.03- 0.03)	U (0, b)	0.09 (0.03-0.15)

Model 1: no informative priors

Model 2: informative prior on prevalence of cervical conductive disturbance (mode 60%; 5th percentile= 10%)

Model 3: informative priors on prevalence and Se_{RX} (mode 50%; 5th percentile 10%) and Sp_{RX} (mode 70%; 5th percentile = 40%)

DISCUSSION

This study brought novelty to equine neurology in two ways. Not only was it the first study to evaluate TMS in a large population, it also is the first evaluation of available diagnostic tests for spinal cord dysfunction taking into account the absence of a gold standard.

TMS-MMEP was the best test to detect spinal cord dysfunction in horses and had the highest specificity. The neurological examination was second best and had the highest sensitivity. The accuracy of cervical radiography, especially the sensitivity (40-50%), was poor, as also reported in previous research (Janes et al., 2014; Levine et al., 2007; Papageorges et al., 1987; Tomizawa et al., 1994). This low accuracy can be explained by some limitations of the study. First, spinal cord diseases like equine herpesvirus myeloencephalitis and EDM/NAD, spinal cord compression caused by soft tissue or thoracic or lumbar lesions will not be visible on cervical radiographs, while these will cause abnormalities on the neurological examination and probably also on TMS-MMEP. Second, enlarged articular process joints can also cause spinal cord compression, but as they are also common in normal horses without neurological deficits (Down and Henson, 2009), they were not included in this study. And third, the sensitivity will be influenced by the chosen cut-off values. In the present study, the 0.485 cut-off suggested by Hahn et al. (2008) was used. In this study, there were no false positives and spinal cord disease was confirmed with histopathology. Earlier, Moore et al. (1994) suggested to use a sagittal ratio of 0.52 for C4-C5 and 0.56 for C7. Logically, using these cut-off values, the sensitivity will increase but also the rate of false positives will increase. For example for C4, 8 out of 137 horses were considered positive, 3 of them were ataxic, but 5 were just normal control horses and thus false positives. By using a lower cut-off, the rate of false positives could be strongly reduced. However, the results of the present study indicate that cervical radiography is the least interesting test for diagnosis of spinal cord disease. Moreover, because of the low sensitivity, the question may rise whether cervical radiographs should still be taken, especially given exposure of horse, owner and veterinarian to radiation. For identification of the lesion diagnostic imaging is required, but myelography or CT might be a better option.

Concerning TMS-MMEP, several decision criteria were tested. Similar to human medicine, minimal latency time delivered a higher overall accuracy for TMS-MMEP than the mean values. However, by using the mean latency time, a higher sensitivity of MMEP could be achieved. So, the choice for minimal or mean latency time may, in the future, vary depending on the purpose of the diagnostic test. For screening purposes, requiring a high sensitivity, mean latency times are the better option, while if confirmation of spinal cord disease is wanted, a high specificity is needed making the minimal latency time more suitable. Furthermore, the accuracy was better for the pelvic than for the thoracic limbs. Decision making based on the thoracic limbs alone or if both thoracic and pelvic limb latency times have to be prolonged, does not seem interesting.

Concerning the neurological examination, a limitation was that horses with grade 1 were also considered normal in the present study. This decision was based on the fact that certainly in mild cases, the observer agreement about the presence of neurological abnormalities might be poor (Olsen et al., 2014; Saville et al., 2017). Therefore, caution should be taken when taking decisions based on the clinical examination, especially when signs are subtle (Olsen et al., 2014) or when orthopedic disease is present. As the study population also included horses suspected of having orthopedic disease and a positive diagnosis of neurological disease might have a serious impact, the authors chose to give the horses with grade 1 ataxia the benefit of the doubt. By considering horses with grade 1 abnormal, the sensitivity of the neurological examination to detect spinal cord dysfunction will increase but the specificity will decrease.

In conclusion, this study showed that TMS-MMEP, using the minimal or in the second place the mean latency time of the pelvic limbs, is the best diagnostic test to diagnose spinal cord disease in a population of horses admitted with suspected ataxia/lameness or purchase control. The neurological examination was the second best diagnostic test and had the highest sensitivity. The accuracy of the cervical radiography was low. Therefore, the authors suggest to screen horses with the neurological examination and to confirm spinal cord disease using TMS-MMEP. Based on this outcome, decisions can be taken concerning further examinations to find the exact etiology of disease but, as the accuracy of the cervical radiographs was low, other imaging techniques such as myelography or CT might be a better choice.

REFERENCES

Aleman, M., Finno, C.J., Higgins, R.J., Puschner, B., Gericota, B., Gohil, K., LeCouteur, R.A., Madigan, J.E., 2011. Evaluation of epidemiological, clinical, and pathological features of neuroaxonal dystrophy in Quarter Horses. Journal of the American Veterinary Medical Association 239, 823-833.

Burns, E.N., Finno, C.J., 2018. Equine degenerative myeloencephalopathy: prevalence, impact, and management. Veterinary medicine (Auckland, N.Z.) 9, 63-67.

Down, S.S., Henson, F.M., 2009. Radiographic retrospective study of the caudal cervical articular process joints in the horse. Equine Veterinary Journal 41, 518-524.

Finno, C.J., Famula, T., Aleman, M., Higgins, R.J., Madigan, J.E., Bannasch, D.L., 2013. Pedigree analysis and exclusion of alpha-tocopherol transfer protein (TTPA) as a candidate gene for neuroaxonal dystrophy in the American Quarter Horse. Journal of Veterinary Internal Medicine 27, 177-185.

Hahn, C.N., Handel, I., Green, S.L., Bronsvoort, M.B., Mayhew, I.G., 2008. Assessment of the utility of using intra- and intervertebral minimum sagittal diameter ratios in the diagnosis of cervical vertebral malformation in horses. Veterinary Radiology and Ultrasound 49, 1-6.

Janes, J.G., Garrett, K.S., McQuerry, K.J., Pease, A.P., Williams, N.M., Reed, S.M., MacLeod, J.N., 2014. Comparison of magnetic resonance imaging with standing cervical radiographs for evaluation of vertebral canal stenosis in equine cervical stenotic myelopathy. Equine Veterinary Journal 46, 681-686.

Levine, J.M., Adam, E., MacKay, R.J., Walker, M.A., Frederick, J.D., Cohen, N.D., 2007. Confirmed and presumptive cervical vertebral compressive myelopathy in older horses: A retrospective study (1992-2004). Journal of Veterinary Internal Medicine 21, 812-819.

Levine, J.M., Ngheim, P.P., Levine, G.J., Cohen, N.D., 2008. Associations of sex, breed, and age with cervical vertebral compressive myelopathy in horses: 811 cases (1974-2007). Journal of the American Veterinary Medical Association 233, 1453-1458.

Levine, J.M., Scrivani, P.V., Divers, T.J., Furr, M., Mayhew, I.J., Reed, S., Levine, G.J., Foreman, J.H., Boudreau, C., Credille, B.C., Tennent-Brown, B., Cohen, N.D., 2010. Multicenter case-control study of signalment, diagnostic features, and outcome associated with cervical vertebral malformation-malarticulation in horses. Journal of the American Veterinary Medical Association 237, 812-822.

Mayhew, I.G., 1978. Spinal cord disease in the horse. Cornell Veterinarian, inc., Ithaca, N.Y.

Mayhew, I.G., 2008. Large Animal Neurology - neurological evaluation form, 2nd ed. Wiley-Blackwell, Chichester.

Moore, B.R., Reed, S.M., Biller, D.S., Kohn, C.W., Weisbrode, S.E., 1994. Assessment of vertebral canal diameter and bony malformations of the cervical part of the spine in horses with cervical stenotic myelopathy. American Journal of Veterinary Research 55, 5-13.

Nollet, H., Deprez, P., Van Ham, L., Verschooten, F., Vanderstraeten, G., 2002. The use of magnetic motor evoked potentials in horses with cervical spinal cord disease. Equine Veterinary Journal 34, 156-163.

Nollet, H., Deprez, R., Van Ham, L., Dewulf, J., Decleir, A., Vanderstraeten, G., 2004. Transcranial magnetic stimulation: normal values of magnetic motor evoked potentials in 84 normal horses and influence of height, weight, age and sex. Equine Veterinary Journal 36, 51-57.

Nollet, H., Van Ham, L., Dewulf, J., Vanderstraeten, G., Deprez, P., 2003a. Standardization of transcranial magnetic stimulation in the horse. Veterinary Journal 166, 244-250.

Nollet, H., Van Ham, L., Verschooten, F., Vanderstraeten, G., Deprez, P., 2003b. Use of magnetic motor-evoked potentials in horses with bilateral hind limb ataxia. American Journal of Veterinary Research 64, 1382-1386.

Nollet, H., Vanschandevijl, K., Van Ham, L., Vanderstraeten, G., Deprez, P., 2005. Role of transcranial magnetic stimulation in differentiating motor nervous tract disorders from other causes of recumbency in four horses and one donkey. Veterinary Record 157, 656-658.

Olsen, E., Dunkel, B., Barker, W.H., Finding, E.J., Perkins, J.D., Witte, T.H., Yates, L.J., Andersen, P.H., Baiker, K., Piercy, R.J., 2014. Rater agreement on gait assessment during neurologic examination of horses. Journal of Veterinary Internal Medicine 28, 630-638.

Papageorges, M., Gavin, P.R., Sande, R.D., Barbee, D.D., Grant, B.D., 1987. Radiographic and Myelographic Examination of the Cervical Vertebral Column in 306 Ataxic Horses. Veterinary Radiology 28, 53-59.

Rijckaert, J., Pardon, B., Van Ham, L., van Loon, G., Deprez, P., 2018. Magnetic Motor Evoked Potential Recording in Horses Using Intramuscular Needle Electrodes and Surface Electrodes. Journal of Equine Veterinary Science 68, 101-107.

Rijckaert, J., Pardon, B., Saey, V., Raes, E., Van Ham, L., Ducatelle, R., van Loon, G., Deprez, P. Determination of magnetic motor evoked potential latency time cut-off values for detection of spinal cord dysfunction in horses Journal of Veterinary Internal Medicine. Accepted june 2019

Saville, W.J.A., Reed, S.M., Dubey, J.P., Granstrom, D.E., Morley, P.S., Hinchcliff, K.W., Kohn, C.W., Wittum, T.E., Workman, J.D., 2017. Interobserver Variation in the Diagnosis of Neurologic Abnormalities in the Horse. Journal of Veterinary Internal Medicine 31, 1871-1876.

Tomizawa, N., Nishimura, R., Sasaki, N., Nakayama, H., Kadosawa, T., Senba, H., Takeuchi, A., 1994. Relationships between radiography of cervical vertebrae and histopathology of the cervical cord in wobbling 19 foals. The Journal of veterinary medical science / the Japanese Society of Veterinary Science 56, 227-233.

Tyler, C.M., Davis, R.E., Begg, A.P., Hutchins, D.R., Hodgson, D.R., 1993. A survey of neurological diseases in horses. Australian Veterinary Journal 70, 445-449.

van Biervliet, J., Scrivani, P.V., Divers, T.J., Erb, H.N., de Lahunta, A., Nixon, A., 2004. Evaluation of decision criteria for detection of spinal cord compression based on cervical myelography in horses: 38 cases (1981-2001). Equine Veterinary Journal 36, 14-20.

CHAPTER 8

GENERAL DISCUSSION

Former research concerning TMS in horses provided a workable protocol for performing TMS in standing horses, making it an attractive method to complement the neurological examination. With the present thesis, the goal was to explore the test in ruminants and to further develop the technique and to prove its value for clinical use in horses.

EXPLORATION IN RUMINANTS

As TMS is a quick and relatively cheap technique, it might be a useful test in cattle. Therefore the technique was introduced and explored in standing and recumbent calves. For this first step exploration, calves were used instead of adult animals as they are more accessible and easier to handle. However, for MMEP recording in calves there were already some difficulties.

Firstly, it was not possible to stimulate the cortex when the coil was placed on the frontal bone of the calves. Transcranial magnetic stimulation did not result in measurable MMEP, probably because the distance between the frontal bone and the brain was too large caused by the presence of the sinuses. An alternative was found in foramen magnum stimulation.

Secondly, in the standing calves, responses could be recorded easily. In the recumbent calves, however, this was not the case. In only 2/3 of the perfectly normal calves, MMEP could be recorded. Of course this is an important limitation. As the false positive rate in the normal calves was really high, the test is not able to make a distinction between neurologically normal or abnormal in recumbent animals. A negative test (= a normal result) in a recumbent animal, probably can be used to confirm a normal nervous system, but a positive test does not provide any certainty. Furthermore, as MMEP were only gathered in normal animals, conclusions about the ability of diagnosing nervous tract disease cannot be made from this thesis. However, in clinical circumstances, abnormal MMEP in ruminants are often confirmed by medical imaging or pathology afterwards.

Finally, the test is not well tolerated in unsedated calves. Especially the placement of the intramuscular needle electrodes might evoke adverse reactions in the animals. These reactions cause dislodging of the electrodes requiring multiple replacements. The use of adhesive surface electrodes might be a solution, but, this was not the only issue. After a couple of stimulations, the calves refused more and more to keep their head in the optimal position. If the 90° angle between head and dorsal neck cannot be achieved, stimulation and MMEPs are inappropriate. Sedating the calves could resolve both issues at once. Unfortunately, in calves sedation really influenced MMEPs significantly. Sedation protocols with acepromazine, xylazine and detomidine have been investigated preliminary, but none of these protocols resulted in reliable MMEP responses. Best responses could be recorded with acepromazine, but its use is prohibited in cattle. Xylazine is known to cause muscle relaxation and in relaxed muscles, it is difficult to register MMEPs. However, in dogs sedation with xylazine still allowed recording of MMEPs, while the dosage is 10x higher than in cattle (Van Ham et al., 1994). Also (me)detomidine had little or no influence on MMEPs in dogs (Van Ham et al., 1994) and horses (Nollet et al., 2003a), while it did influence MMEPs in calves.

So, in conclusion, TMS-MMEP is possible in calves, but important limitations for its practical use were identified.

FURTHER IMPROVEMENT OF TMS-MMEP IN HORSES

For further development and improving TMS in horses, comparison was made with TMS in human medicine, which is far more developed. However, there are a lot of differences between TMS in humans and horses which cannot be overcome. Factors to which great importance is attached in humane medicine, are just not possible to control in horses.

First, the facilitation principle: demanding a certain percentage of maximal muscle contraction will never be possible in horses. Yet, it is tried to standardize the pre-stimulation muscle activity by assuring the horse is standing relaxed and square, weight baring on its 4 feet. In this way, there will always be a certain level of muscle activity in the postural muscles but the exact level cannot be controlled.

Second, in human medicine central motor conduction time (CMCT), and not cortex to muscle time, is measured to evaluate spinal cord function. In animals, this parameter has never been determined. The best way to calculate CMCT is by using the F wave method as described before. As this strong electrical stimulation is painful, it will only be possible in horses under general anaesthesia. But then, it is no longer the quick, cheap and non-invasive test we are searching for. The second and less precise technique to estimate the peripheral conduction time was by stimulation over the vertebral column. Again, electrical stimulation will only be possible under general anaesthesia and for magnetic stimulation, the distance between stimulation site and spinal cord will probably too large for reliable muscle responses. Therefore, in animals, the choice has been made to use cortex to muscle time. So, delayed latency times can reflect or a central or peripheral lesion. However, peripheral lesions are not that common in horses. Anyway, in case of abnormal responses, it should always be taken into account that peripheral lesions might be involved.

Other factors with a minimizing effect on MMEP variation have been introduced, analogous to human medicine. Nowadays, registration of MMEPs in left and right limb are performed simultaneously. Like this, variation between left and right cannot be contributed to inter stimulus variations and comparison between left and right is more reliable. Also, the former standard intramuscular needle electrodes have been replaced by surface electrodes for MMEP recordings.

The surface electrodes were much better tolerated by the horses and dislodged less frequently than the intramuscular needle electrodes. Surface electrodes measure a summed muscle activity of many motor neurons while the intramuscular recordings only measure the activity of a few muscle fibres, but despite these differences, latency time values were similar for both electrode types. The latency time values in chapter 5 for both electrode types, differ from other reported normal values in horses. As already suggested in the discussion over there, probably some mildly neurologically affected horses were present in the test population. On the other hand, the 95% reference intervals do not seem so abnormal in our experience. Nowadays, in clinical circumstances often latency times of 18-20 ms in thoracic and 38-40 ms in pelvic limbs are found in clinically normal horses. One reason can be the size of the horses. Mayhew and Washbourne (1996) only used ponies and the normal values of Nollet et al. (2004) were also based on horses with a mean withers height of 138 cm and a

mean weight of 383 kg. The horses we used had a mean withers height of 160 and a mean weight of 535 kg. As height has an influence on latency time, this difference may not be neglected. A second influencing factor might be the subjective determination of latency time. Looking at the analyses of Nollet and colleagues (Nollet et al., 2002a; Nollet et al., 2004; Nollet et al., 2003b) and comparing these with our analyses, the determination of onset latency seems similar in the thoracic limbs, but differs for the pelvic limbs. Nollet estimated the onset of the muscle contraction easily 4-5 ms earlier compared to what was done in this thesis (Figure 1). Which of both is chosen for MMEP analysis does not really matter as long it is always the same technique and the matching reference values are used.

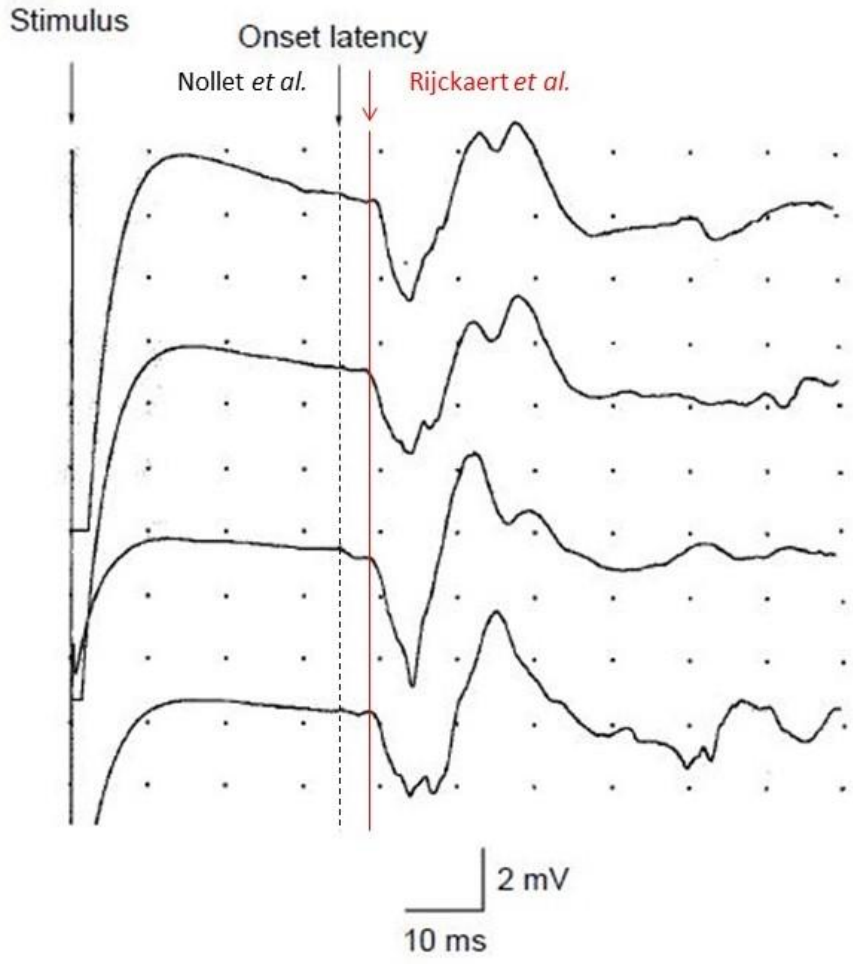


Figure 1.. Different determination by different observers of onset latency of the pelvic limbs. (adapted from Nollet et al. (2002a))

In contrast to latency time, amplitude values differed between surface and intramuscular needle measurement. This is perfectly logical as every little displacement of the needle will result in registration of activity of different fibres with different MMEP shape. However, as latency time has always been the most informative and reliable parameter for MMEPs, this difference in amplitude was considered not to be a limitation for the use of surface electrodes.

As in human medicine (Ertekin et al., 1998), also paravertebral MMEP measurements have been introduced with the goal to enable more precise lesion localization in the future. The most interesting aspect of this cervical MMEP technique is the ability to evaluate the function of the motor nervous tract. (Advanced) diagnostic imaging provides a lot of information, but might also show subclinical lesions (da Costa et al., 2006; De Decker et al., 2011). By combining neurophysiological testing with diagnostic imaging findings, the clinical importance of imaging abnormalities can be determined. The present study only includes information about normal control horses. Whether cervical MMEPs are really able to localize lesions needs to be determined in ataxic horses in future, but the first trials seem promising. Moreover, besides cervical muscle MMEPs, also thoracic and lumbar muscle MMEPs offer perspectives for future research.

In conclusion, TMS in horses remains a simplified version of TMS in human medicine, measuring different parameters and providing different information. Also, normal values vary between studies and cut-off values to diagnose spinal cord disease are not available, therefore, complete validation was necessary.

VALIDATION OF TMS-MMEP IN HORSES

Previous research already demonstrated the use of TMS in horses and suggested it as a diagnostic test for spinal cord disease (Nollet et al., 2002a; Nollet et al., 2003b; Nollet et al., 2005). However, reliable cut-off values based on histopathology and sensitivity and specificity calculations were not available yet. Therefore, the present research made an important contribution to the validation of TMS-MMEP. Cut-off values were calculated based on histopathological findings (chapter 6) and these were validated afterwards on a larger number of cases using Bayesian latent class modelling to account for the absence of a gold standard (chapter 7).

The cut-off for the thoracic limbs was set at 22 ms, but for the pelvic limbs, actually 2 different cut-offs were proposed, depending on the selected cases. If only horses with confirmed compressive lesions were included, the optimal pelvic latency time cut-off was 43 ms. If horses with very mild ataxia and minor histopathological or solely inflammatory lesions were included, this cut-off decreased to 40 ms. Using the 40 ms cut-off, TMS-MMEP correctly identified 16/17 horses as case and 5/5 as control horses. The 1 horse considered false negative on TMS-MMEP, had a grade 1 ataxia and very subtle degenerative and inflammatory lesions on histopathology. So, it is possible that this one horse actually had an orthopaedic problem instead of neurological issues and that it actually should be considered as a control horse. Using the 43 ms cut-off resulted in a second false negative horse on TMS-MMEP. This second horse showed a grade 4 ataxia but had no signs of compression on medical imaging and only mild inflammatory lesions on histopathology. As there was no 100% confirmation of spinal cord disease in these cases and because the cut-offs needed to be based on confirmed cases, it was decided to eliminate these 2 more doubtful cases and to use 43ms as cut-off in the following study. However, the 40 ms might be interesting to consider as an alternative and would also be interesting to evaluate on a large number of horses.

Using 40 ms, the sensitivity of TMS-MMEP will, and the chance of identifying inflammatory caused spinal cord disease might even increase then, as it seems that inflammatory spinal cord disease prolongs latency time less than compressive spinal cord disease. However, whether TMS-MMEP latency time is always abnormal in inflammatory spinal cord disease is unsure. Nollet et al. (2002b) described 2 cases diagnosed with equine herves virus. One myeloencephalitis showed abnormal latency times, but the other had normal latency times at the time of the examination. At that time, the horse had already improved clinically, so, it is not totally clear if TMS did not detect the disease or if the spinal cord function really returned normal again at that point. Also in humans, motor conduction can be prolonged in cases with acute myelitis but a normal conduction is possible too (Kalita and Misra, 2000), while in cases with compressive myelopathy, motor conduction is abnormal in almost all patients (Chen et al., 2008; Nardone et al., 2014). Therefore, it can be concluded that TMS-MMEP has great potential to detect spinal cord compression, but for other neurological diseases, the accuracy still needs to be determined.

Furthermore, as ataxia is per definition a sensory dysfunction while TMS-MMEP determines the motor conduction through the spinal cord, it is possible that horses show spinal ataxia but have normal TMS-MMEP latency times. In compressive myelopathy, often both, sensory and motor tracts, are affected but it is possible in mild cases that only the sensory nerves, who are positioned on the outside of the spinal cord, are damaged. However, in the validation study (chapter 7), no attention was paid to the etiology of ataxia. Despite the fact that the study probably also contained horses with inflammatory lesions, purely sensory lesions or neuroaxonal dystrophy/equine degenerative myelopathy, TMS-MMEP had the highest accuracy. If only horses with spinal cord compression would have been included, probably accuracy would have been even higher.

Pelvic limb latency times had a higher sensitivity for spinal cord dysfunction diagnosis than thoracic latency times. This is also noticed in human medicine (Deftereos et al., 2009; Kalita and Misra, 2000) and in dogs (da Costa et al., 2006; De Decker et al., 2011). Da Costa et al. (2006) suggested this could be a consequence of the caudally located cervical compression in their dogs, but it is also suggested that the lateral positioned motor tracts, being those to the pelvic limbs, are affected first in cases of spinal compression. As the motor tracts to the thoracic limbs are positioned more medially, the effect of spinal cord compression will be less obvious than in the pelvic limbs (De Decker et al., 2011). Furthermore, in chapter 7, the lesion localisation was not taken into account in the case selection. So, the study population will also include cases with spinal cord lesions caudal to the thoracic limbs. Furthermore, the highest accuracy for TMS-MMEP as a diagnostic test was found using the minimal latency time values instead of the in horses routinely used mean values. In human medicine, it is standard procedure to use minimum latency times (Chan et al., 1998; Funaba et al., 2015). Though, Nakamae et al. (2010) also superimposed 4 responses to measure a mean latency time, similar to Nollet et al. (2004) on which all the research in the present doctoral thesis was based. However, since the influence of using minimal latency time instead of mean latency time is determined now, a switch should be considered. However, choosing mean or minimal latency time might depend on the goal of the examination. Using the minimal values, TMS-MMEP is more specific while using the mean values, the test is more sensitive.

Cervical radiography had a low accuracy, a lot lower than TMS-MMEP, for detection of spinal cord disease. This could be expected as thoracic or lumbar compression, soft tissue compression, inflammatory diseases and neuroaxonal dystrophy/equine degenerative myelopathy will not be visible. Moreover, even to detect cervical vertebral stenotic myelopathy, cervical radiography is known to have low sensitivity and only moderate specificity (Levine et al., 2007; Levine et al., 2010). So, the question rises whether cervical radiographs are useful for diagnosis of CVM. Certainly in cases where TMS-MMEP is negative, it is very unlikely to detect abnormalities on the radiographs or even on the myelogram. In cases with a positive TMS-MMEP, diagnostic imaging is still needed to confirm the etiology of the ataxia and to localize the site of the lesion for treatment options and prognosis. However, in these cases myelography or contrast CT are probably better options.

RECOMMENDATIONS FOR USE OF TMS-MMEP IN PRACTICE

A diagnostic approach to spinal cord disease is suggested based on the results of the present research project (Figure 2). Because of its high sensitivity, the neurological examination is most suitable to be used as screening test for neurological disease. This first screening can be performed by a veterinary practitioner in the field. If there is any doubt about the presence of a possibly neurological gait abnormality, the horse is sent to the TMS-MMEP examination, where the minimal pelvic latency time, with a cut-off value of 43ms, should be used as decision threshold. Depending on the results of the different tests, different decisions concerning further examinations or purchase might be made.

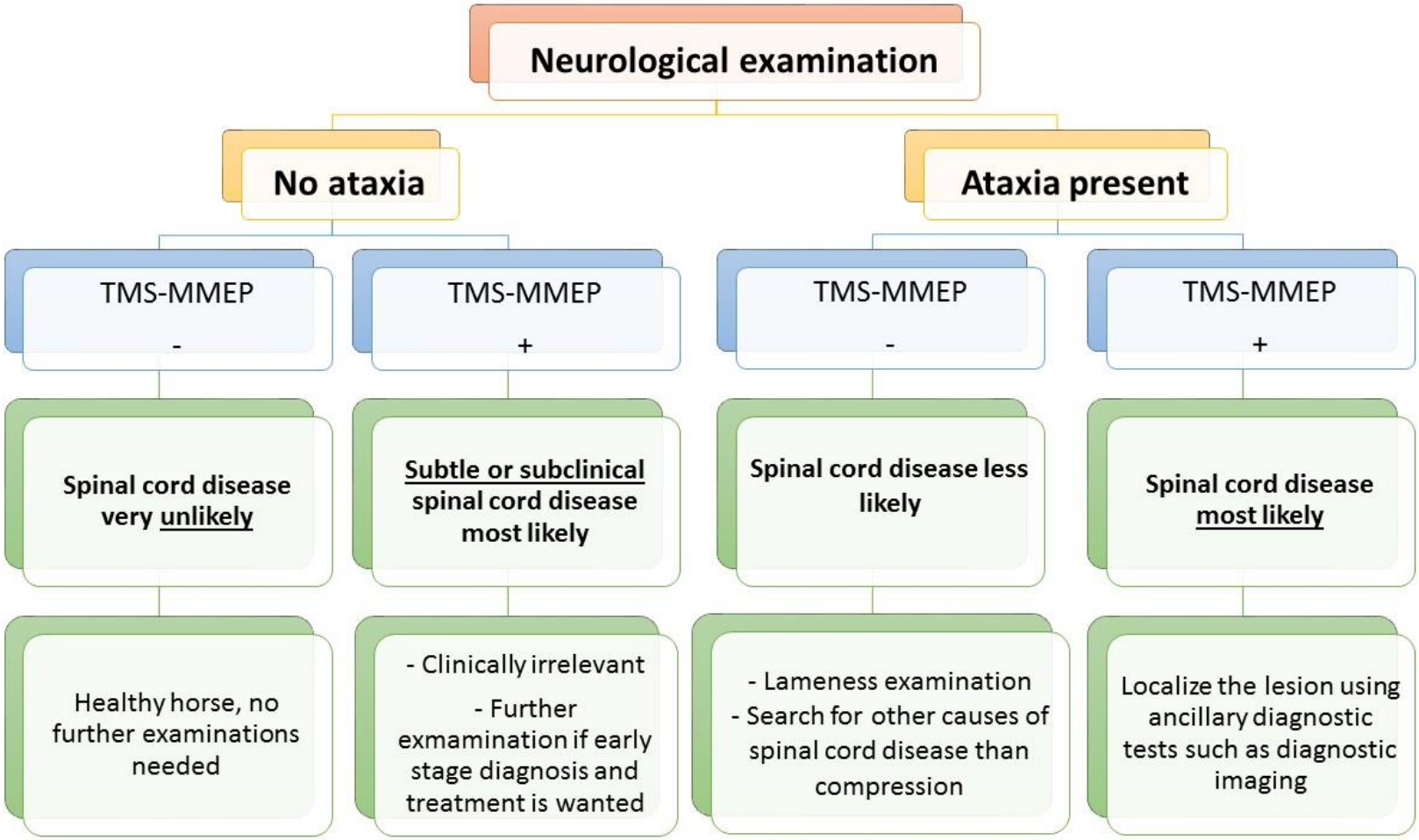


Figure 2. Diagnostic approach to confirm or exclude spinal cord disease in horses, including TMS-MMEP examination. "+" indicates an abnormal (=positive) test result, "-" indicates a normal (=negative) test result. Disagreement between the neurological examination and TMS-MMEP (two middle columns) is not frequently seen.

To demonstrate the value of TMS-MMEP in this approach, Fagan nomograms can be used to translate sensitivity and specificity to probability of disease. This nomogram combines the pre-test probability of disease with a likelihood ratio (LR(Positive LR= sensitivity/(1-specificity), negative LR= (1-sensitivity)/specificity)) to end with a post test probability of disease. The higher the positive and the lower the negative likelihood ratio, the bigger the difference between pre and post test probabiltiy and the better the diagnostic test (Caraguel and Vanderstichel, 2013).

For example, TMS-MMEP had a sensitivity of 87.5% and a specifity of 97.4% resulting in a positive LR of 34 and a negative likelihood ratio of 0.13. Using these values in our test population with a spinal cord disease probability of about 50%, the chance of spinal cord disease is about 97% in case of a positive TMS-MMEP test. In case of a negative TMS-MMEP test, there is still about 12% chance spinal cord disease is present (Figure 3). If the neurological examination (sensitivity 97.6% and specificity of 74.7%) and TMS-MMEP are combined in serial testing, the certainty about the presence of disease will even increase. The negative LR increases a little to 0.15 but the positive LR increases significantly to 122. This results in more than 99% chance of spinal cord disease in case of a positive test and only 13% chance of disease in case of a negative test result. However, if a higher sensitivity is required, for instance in prepurchase examinations, the tests can be used in parallel to achieve a sensitivity of 99.7% and a specificity of 72.8%. Using this parallel test protocol, there is virtually 0% chance of disease in case of a negative test. However, if the test is positive, there is 21% chance that the horse does not have spinal cord disease anyway (Figure 4).

Based on the post-test probabilities, owners can make decisions about further actions concerning their horse more easily. Some owners will not do extra costs, while others want to know the exact etiology and want to discuss treatment options and prognosis. In that case, medical imaging is the logical next step in most cases. However, in the future, this next step might become cervical muscle MMEP recording. In case of a negative test in an ataxic horse, it might be indicated to perform a profound lameness examination or to search for another etiology than spinal cord compression. If TMS-MMEP is negative in a horse without ataxia, the horse has a very high chance to be totally normal.

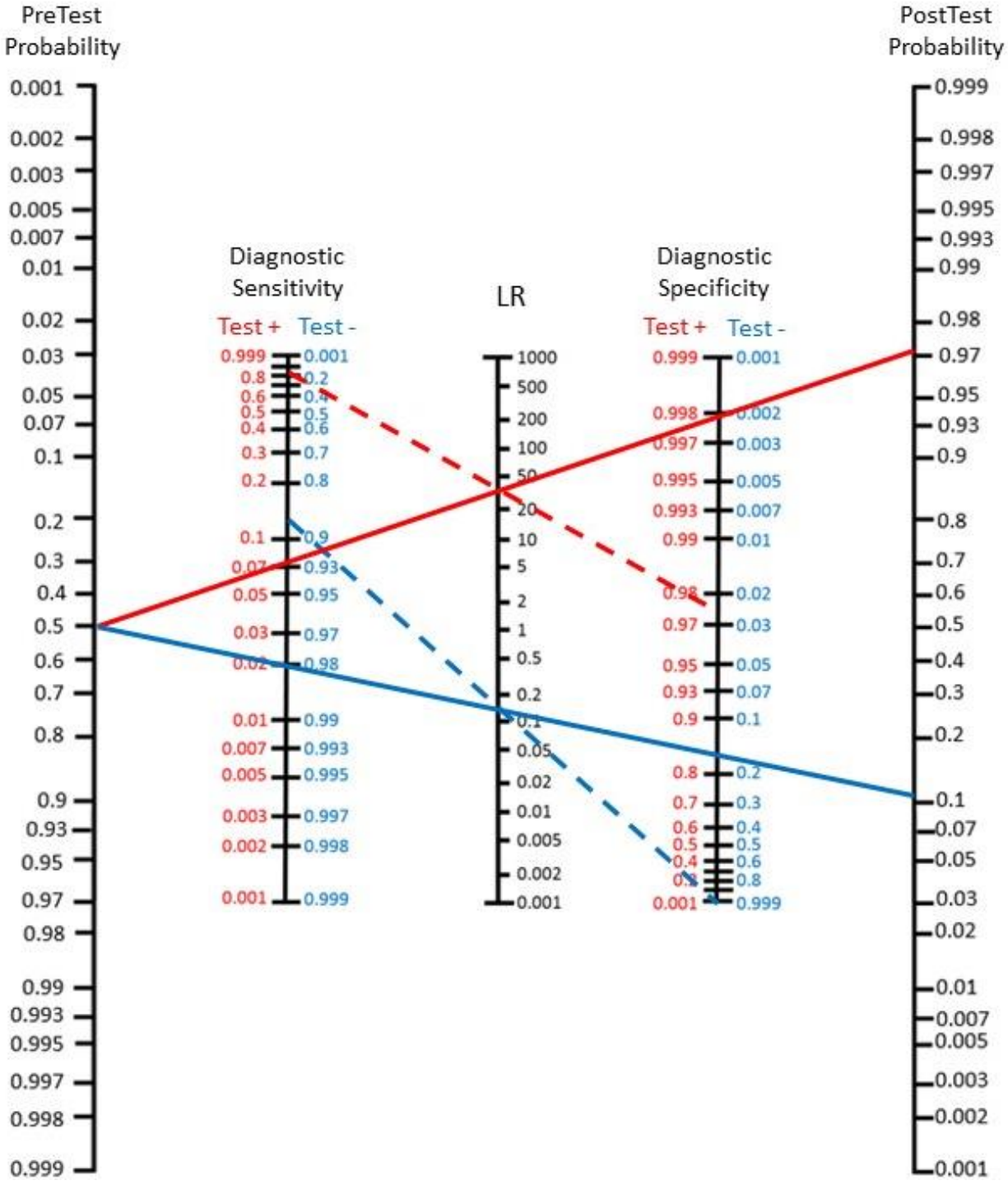


Figure 3. Fagan nomogram to demonstrate the added value of TMS-MMEP to diagnose spinal cord disease using 43 ms as cut-off for minimum latency time for the pelvic limbs. Likelihoodratio determination is shown by the dashed lines, the full lines represent the difference between pre- and post-test probability of spinal cord dysfunction. The red lines represent a positive test result, the blue lines represent a negative test result.

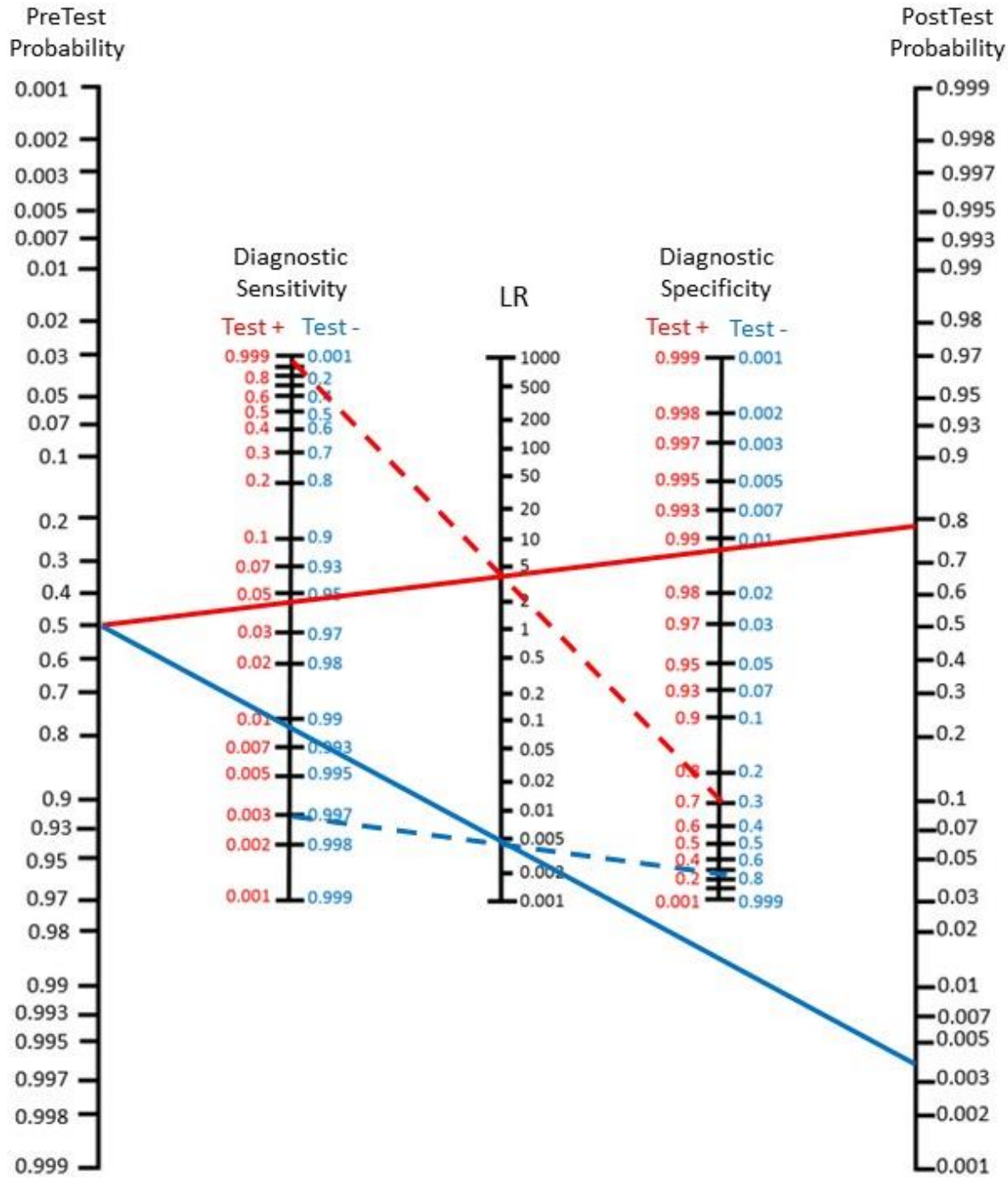


Figure 4. Fagan nomogram to demonstrate the added value of parallel testing of the neurological examination and TMS-MMEP to diagnose spinal cord disease using 43 ms as cut-off for minimum latency time for the pelvic limbs. Likelihoodratio determination is shown by the dashed lines, the full lines represent the difference between pre- and post-test probability of spinal cord dysfunction. The red lines represent a positive test result, the blue lines represent a negative test result.

FUTURE RESEARCH

Despite the fact that this thesis demonstrates the value of TMS-MMEP to diagnose spinal cord disease in horses, several questions remain unanswered and many new research opportunities became clear. First, it is not known whether TMS-MMEP is able to make a differentiation in the etiology of the spinal cord disease. To date, it only confirms spinal cord disease. Second, TMS-MMEP is only validated as a diagnostic test, whereas its value for prognosis and therapy selection is currently undiscovered. Third, in case of abnormal MMEP latency times, one lesion is suspected, but what if multiple compression sites are present or peripheral nerves contribute to the clinical signs. Also, more precisely localization of lesions is wanted. Fourth, in the present study, 1 latency time cut-off value is suggested, regardless of the height of the horses, while height does influence MMEP latency time. In our horse population, the majority of the horses presented with ataxia are large warmblood horses, but in smaller breeds this suggested cut-off might not be appropriate. And last, in the present research, the focus was put on latency time, as this is the most reliable parameter for corticospinal integrity (Groppa et al., 2012). However, it is still interesting to look at amplitude too, as low amplitude MMEPs with little delay or absence of responses suggest neuronal or axonal loss (Nardone et al., 2015; Nardone et al., 2014).

Therefore, it would be, in the first place, interesting to validate the cervical muscle TMS-MMEP in horses and to even continue with thoracic and lumbar muscle TMS-MMEP to enable functional lesion localisation. Certainly these more caudal regions are interesting for further examination as they are difficult to evaluate using diagnostic imaging because of the size of the horses. Furthermore, the effect of articular process joints enlargements should be investigated in correlation with clinical signs and diagnostic imaging. At this moment, these articular process joints are believed to cause problems in sport horses but they are difficult to examine as they might be enlarged without clinical impact in older horses (Down and Henson, 2009). So, to date, the clinical importance of these enlargements is not known in horses. In dogs, it was already suggested that TMS-MMEP could be useful to discriminate between clinically relevant and irrelevant spinal cord compression on diagnostic imaging (De Decker et al., 2011). So, maybe TMS-MMEP might help to do so in horses too. Furthermore, it should be investigated if it is possible to make a differentiation in the etiology of spinal cord disease using TMS-MMEP. Therefore, maybe amplitude should be involved again. However, before it

can be used reliably, the variation should be decreased. The use of surface electrodes was a successful first step, but maybe also the option to use the compound muscle action potential should be investigated.

Concerning TMS-MMEP in ruminants, the influence of other diseases causing weakness or recumbency (severe diarrhoea with electrolyte imbalances, hypocalcemia, encephalitis,…) on MMEPs should be investigated and before the test can be used in adult ruminants, reference values should be determined as the results from the calves cannot be extrapolated.

CONCLUSION

This thesis has contributed to the field of large animal but especially equine neurology by demonstrating the high accuracy of TMS-MMEP to diagnose spinal cord dysfunction. The test is easy to perform, well tolerated, non-invasive and has a very high specificity and sensitivity, both a lot higher than for cervical radiography. By using this TMS-MMEP more routinely, diagnosis of spinal cord dysfunction can be simplified and owners will have a higher level of certainty concerning the presence of spinal cord disease in their horses.

REFERENCES

Caraguel, C.G., Vanderstichel, R., 2013. The two-step Fagan's nomogram: ad hoc interpretation of a diagnostic test result without calculation. Evidence Based Medicine 18, 125-128

Chan, K.M., Nasathurai, S., Chavin, J.M., Brown, W.F., 1998. The usefulness of central motor conduction studies in the localization of cord involvement in cervical spondylytic myelopathy. Muscle & nerve 21, 1220-1223.

Chen, R., Cros, D., Curra, A., Di Lazzaro, V., Lefaucheur, J.P., Magistris, M.R., Mills, K., Rosler, K.M., Triggs, W.J., Ugawa, Y., Ziemann, U., 2008. The clinical diagnostic utility of transcranial magnetic stimulation: report of an IFCN committee. Clinical Neurophysiology119, 504-532.

da Costa, R.C., Poma, R., Parent, J.M., Partlow, G., Monteith, G., 2006. Correlation of motor evoked potentials with magnetic resonance imaging and neurologic findings in Doberman Pinschers with and without signs of cervical spondylomyelopathy. American Journal of Veterinary Research 67, 1613-1620.

De Decker, S., Van Soens, I., Duchateau, L., Gielen, I.M.V.L., van Bree, H.J.J., Binst, D.H.A.R., Waelbers, T., Van Ham, L.M.L.M., 2011. Transcranial magnetic stimulation in Doberman Pinschers with clinically relevant and clinically irrelevant spinal cord compression on magnetic resonance imaging. Journal of the American Veterinary Medical Association 238, 81-88.

Deftereos, S.N., Kechagias, E.A., Panagopoulos, G., Seretis, A., Orphanidis, G., Antoniou, E., Georgakoulias, N., Karageorgou, C.E., 2009. Localisation of cervical spinal cord compression by TMS and MRI. Functional neurology 24, 99-105.

Down, S.S., Henson, F.M., 2009. Radiographic retrospective study of the caudal cervical articular process joints in the horse. Equine Veterinary Journal 41, 518-524.

Ertekin, C., Uludag, B., On, A., Yetimalar, Y., Ertas, M., Colakoglu, Z., Arac, N., 1998. Motor-evoked potentials from various levels of paravertebral muscles in normal subjects and in patients with focal lesions of the spinal cord. Spine 23, 1016-1022.

Funaba, M., Kanchiku, T., Imajo, Y., Suzuki, H., Yoshida, Y., Nishida, N., Taguchi, T., 2015. Transcranial magnetic stimulation in the diagnosis of cervical compressive myelopathy: comparison with spinal cord evoked potentials. Spine 40, 161-167.

Groppa, S., Oliviero, A., Eisen, A., Quartarone, A., Cohen, L.G., Mall, V., Kaelin-Lang, A., Mima, T., Rossi, S., Thickbroom, G.W., Rossini, P.M., Ziemann, U., Valls-Sole, J., Siebner, H.R., 2012. A practical guide to diagnostic transcranial magnetic stimulation: report of an IFCN committee. Clinical Neurophysiology123, 858-882.

Kalita, J., Misra, U.K., 2000. Neurophysiological studies in acute transverse myelitis. Journal of Neurology 247, 943-948.

Levine, J.M., Adam, E., MacKay, R.J., Walker, M.A., Frederick, J.D., Cohen, N.D., 2007. Confirmed and presumptive cervical vertebral compressive myelopathy in older horses: A retrospective study (1992-2004). Journal of Veterinary Internal Medicine 21, 812-819.

Levine, J.M., Scrivani, P.V., Divers, T.J., Furr, M., Mayhew, I.J., Reed, S., Levine, G.J., Foreman, J.H., Boudreau, C., Credille, B.C., Tennent-Brown, B., Cohen, N.D., 2010. Multicenter case-control study of signalment, diagnostic features, and outcome associated with cervical vertebral malformation-malarticulation in horses. Journal of the American Veterinary Medical Association 237, 812-822.

Mayhew, I.G., Washbourne, J.R., 1996. Magnetic motor evoked potentials in ponies. Journal of Veterinary Internal Medicine 10, 326-329.

Nakamae, T., Tanaka, N., Nakanishi, K., Fujimoto, Y., Sasaki, H., Kamei, N., Hamasaki, T., Yamada, K., Yamamoto, R., Izumi, B., Ochi, M., 2010. Quantitative assessment of myelopathy patients using motor evoked potentials produced by transcranial magnetic stimulation. European Spine Journal 19, 685-690.

Nardone, R., Holler, Y., Brigo, F., Orioli, A., Tezzon, F., Schwenker, K., Christova, M., Golaszewski, S., Trinka, E., 2015. Descending motor pathways and cortical physiology after spinal cord injury assessed by transcranial magnetic stimulation: a systematic review. Brain research 1619, 139-154.

Nardone, R., Holler, Y., Thomschewski, A., Holler, P., Bergmann, J., Golaszewski, S., Brigo, F., Trinka, E., 2014. Central motor conduction studies in patients with spinal cord disorders: a review. Spinal cord 52, 420-427.

Nollet, H., Deprez, P., Van Ham, L., Verschooten, F., Vanderstraeten, G., 2002a. The use of magnetic motor evoked potentials in horses with cervical spinal cord disease. Equine Veterinary Journal 34, 156-163.

Nollet, H., Deprez, R., Van Ham, L., Dewulf, J., Decleir, A., Vanderstraeten, G., 2004. Transcranial magnetic stimulation: normal values of magnetic motor evoked potentials in 84 normal horses and influence of height, weight, age and sex. Equine Veterinary Journal 36, 51-57.

Nollet, H., Van Ham, L., Gasthuys, F., Dewulf, J., Vanderstraeten, G., Deprez, P., 2003a. Influence of detomidine and buprenorphine on motor-evoked potentials in horses. Veterinary Record 152, 534-537.

Nollet, H., Van Ham, L., Verschooten, F., Vanderstraeten, G., Deprez, P., 2003b. Use of magnetic motor-evoked potentials in horses with bilateral hind limb ataxia. American Journal of Veterinary Research 64, 1382-1386.

Nollet, H., Vanschandevijl, K., Lefere, L., Verschooten, F., Van Ham, L., Ducatelle, R., Vanderstraeten, G., Deprez, P., 2002b. A new neurological examination method in the horse: Magnetic transcranial stimulation. Vlaams Diergeneeskundig Tijdschrift 71, 256-267.

Nollet, H., Vanschandevijl, K., Van Ham, L., Vanderstraeten, G., Deprez, P., 2005. Role of transcranial magnetic stimulation in differentiating motor nervous tract disorders from other causes of recumbency in four horses and one donkey. Veterinary Record 157, 656-658.

Van Ham, L.M., Vanderstraeten, G.G.W., Mattheeuws, D.R.G., Nijs, J., 1994. Transcranial Magnetic Motor Evoked-Potentials in Sedated Dogs. Progress in veterinary neurology 5, 147-154.

SUMMARY

Spinal ataxia, a clinical sign of spinal cord disease, is a common neurological disease in horses. Clinical signs range from subtle to very obvious with recumbency in the worst case. Also in recumbent cattle, spinal cord disease is an important differential diagnosis. Mostly, the diagnosis of spinal cord disease is based on a combination of patient history, neurological examination and diagnostic imaging, if necessary complemented with blood and cerebrospinal fluid analysis. However, sensitivity and specificity of the different tests are rather low and they do not give information about the function of the spinal cord. Therefore, transcranial magnetic stimulation with recording of magnetic motor evoked potentials (TMS-MMEP) has been suggested as an alternative. However, the technique of TMS-MMEP can still be improved, more precise lesion localisation is wanted and, as there is no gold standard for diagnosis of spinal cord disease in horses, the accuracy of the diagnostic test was still unrevealed. Therefore, the present research was performed to provide an answer on these shortcommings.

As a general introduction (Chapter 1), an overview of spinal cord disease in large animals was given, mostly focussed on cervical vertebral myelopathy in horses, as this is most common in Europe. Definition, etiology and pathogenesis, treatment and prognosis, but mainly how to diagnose the disease were discussed. First, the clinical neurological examination was shortly described, second, the different diagnostic imaging techniques, third other diagnostics such as cerebrospinal fluid evaluation, vertebral canal endoscopy and histopathology and last also the electrofysiologic tests electromyography and transcranial magnetic stimulation with recording of magnetic motor evoked potentials (TMS-MMEP). TMS has been used to diagnose spinal cord dysfunction in humans, dogs and in horses, but there are some differences between different species and the accuracy of the test was not determined in horses yet.

Therefore, the aims of the study were (Chapter 2) exploration of the technique in other species and further development and validation of TMS-MMEP as a quick diagnostic test for spinal cord dysfunction in horses. The exploration of the technique in calves was described in chapter 3, the further development of the test in horses was described in chapters 4 and 5, the validation in chapters 6 and 7.

In chapter 3, the useability of TMS-MMEP in other animals, more specifically in normal calves, was investigated. Maximal magnetic stimulation was performed at the level of the foramen magnum in 41 calves, in standing position and lateral recumbency, to determine reference values for onset latency and amplitude. In standing position, clear, reproducible MMEPs were obtained in all calves. In the lateral position, only 64% of the calves showed responses in the four limbs. Age, body weight, height at the withers and rectal temperature had no significant relationship with latency time or amplitude and no left to right differences were noted. Latency time was longer in calves than in horses, possibly due to a different kind of evoked response by foramen magnum stimulation. It was concluded that TMS-MMEP in limb muscles is possible in calves by stimulation at the level of the foramen magnum, but in lateral recumbency, the MMEPs are more difficult to obtain.

In chapter 4, a first step was taken to enable more specific localisation of spinal cord lesions in future. When surgical treatment of cervical vertebral malformation in horses is considered, precise localization of spinal cord compression sites is essential, but challenging. Paravertebral muscle MMEPs have already been used for lesion localization in humans but were unexplored in horses. Therefore, TMS-MMEP was performed on 50 normal horses and muscle responses were recorded at the middle of each cervical vertebra, to evaluate all individual cervical nerves. MMEPs of cervical muscles in normal horses were easy to collect and to evaluate and there was limited intra- and inter-observer variation. It was seen that latency time increased significantly from Cn3 to Cn8, but besides the cervical nerve, also gender and height had an influence on latency time. In the future, these cervical muscle MMEPs should be investigated in ataxic horses to evaluate if lesion localization is possible using this technique.

Chapter 5 describes adhesive surface electrodes as a better tolerated alternative for the formerly used intramuscular needle electrodes. The needle electrodes are painful to place and often dislodge, while the surface electrodes can be placed painless and do not dislodge. However, both measure different things. Surface electrodes register compound muscle potentials that reflect the electrical activity of many motor neurons while intramuscular needle electrodes register MMEPs from a few single muscle fibres. As the normal values in horses were determined using needle electrodes, agreement between both electrode types concerning latency and amplitude measurements needed to be investigated before the switch to surface electrodes could be made. For latency time, a good agreement and repeatability

was found, but for amplitude measurements, the agreement was insufficient. However, as amplitude always has been a less reliable parameter, the better tolerated surface electrodes can be used for TMS-MMEP latency measurements in horses.

The determination of the optimal cut-off values for TMS-MMEP latency time to diagnose spinal cord dysfunction, based on histopathology, was described in chapter 6. TMS-MMEP was already described as a valuable tool to detect neurological dysfunction in horses and increased latency times of MMEPs and ataxia show a good association. However, cut-off values based on confirmed spinal cord dysfunction, were still lacking. Therefore, a case control study with 5 control horses and 17 horses with ataxia was performed. Receiver operating characteristic (ROC)-curve analysis, based on diagnostic imaging, TMS and histopathological findings was used to determine the optimal TMS-MMEP latency time cut-off values for diagnosis of spinal cord dysfunction. Optimal cut-off values were 22 ms in thoracic and 40 ms in pelvic limbs. To detect specifically spinal cord dysfunction caused by compression, the optimal cut-off for thoracic limbs remained 22 ms while it increased to 43 ms in pelvic limbs. Using these threshold values for latency time, a much higher sensitivity and specificity than currently reported for cervical radiography was obtained. These results were promising and merited validation of these cut-offs on a larger dataset, including animals with mild ataxia and gait deficits of orthopaedic nature in future work.

This validation was performed in chapter 7. The accuracy of TMS-MMEP to diagnose spinal cord dysfunction was calculated using bayesian latent class modelling, combining results of neurological examination, cervical radiography and TMS-MMEP of 174 horses admitted at the clinic for neurological examination. In all models, TMS-MMEP was the best test to diagnose spinal cord disease and the neurological examination was second best. Cervical radiography had the lowest accuracy for diagnosis of spinal cord dysfunction. Different models were built for different TMS-MMEP decision criteria, but the best criterion was minimum latency time of the pelvic limbs with a sensitivity of 87.5% and a sensitivity of 97.4%.

In the general discussion (Chapter 8), it was concluded that TMS-MMEP is easy to perform, well tolerated, non-invasive and has a very high accuracy, especially specificity. The test accuracy is a lot better than that from cervical radiography. A diagnostic approach to spinal cord disease was suggested and the effect on the probability of disease in case of a positive or negative TMS-MMEP result was shown using Fagan nomograms. By using this TMS-MMEP more routinely, diagnosis of spinal cord dysfunction can be simplified and owners will have a higher level of certainty concerning the presence of spinal cord disease in their horses. If spinal cord disease is suspected but needs to be confirmed, serial use of the neurological examination and TMS-MMEP would be the optimal strategy. If spinal cord disease needs to be excluded with a very high level of certainty, the neurological examination and TMS-MMEP can be used in parallel.

SAMENVATTING

Spinale ataxie, veroorzaakt door ruggenmerg aandoeningen, is een veel voorkomende neurologische aandoening bij paarden. De klinische tekenen kunnen variëren van zeer subtiel tot zeer ernstig met laterale decubitus in het slechtste geval. Ook bij runderen in laterale decubitus zijn neurologische problemen een belangrijke differentiaaldiagnose. De diagnose van ruggenmerg aandoeningen wordt meestal gesteld op basis van de anamnese, het klinisch neurologisch onderzoek en medische beeldvormingstechnieken. Indien aangewezen, kunnen deze onderzoeken aangevuld worden met bloed- en cerebrospinaal vochtanalyses. Helaas zijn de sensitiviteit en de specificiteit van de verschillende onderzoekstechnieken eerder laag en wordt er geen informatie verkregen over de functionaliteit van het ruggenmerg. Daarom werd transcraniële magnetische stimulatie met registratie van de magnetisch uitgelokte motorische potentialen (TMS-MMEP) vroeger al gesuggereerd als alternatief. Echter, de TMS-MMEP techniek kon nog verbeterd worden en de accuraatheid als diagnostische test was nog niet gekend. Dit onderzoek werd dan ook uitgevoerd met als doel een antwoord te voorzien op deze tekortkomingen.

Als algemene introductie werd een overzicht gegeven betreffende ruggenmerg aandoeningen bij grote huisdieren (Hoofdstuk 1), waarbij vooral werd gefocust op cervicale vertebrale malformatie (CVM) omdat deze het frequentst voorkomt in de Europese warmbloed populatie. De ziekte werd gedefinieerd en de etiologie, pathogenese, behandelingsmogelijkheden, prognose maar voornamelijk ook de diagnose mogelijkheden werden besproken. In de eerste plaats werd het klinisch neurologisch onderzoek kort besproken waarna de medische beeldvorming, andere testen zoals analyse van cerebrospinaal vocht, ruggenmerg kanaal endoscopie en histopathologie en electrofysiologische testen zoals electromyografie en TMS-MMEP volgden. TMS-MMEP werd bij mensen, honden en paarden al gebruikt voor het vaststellen van functiestoornissen ter hoogte van het ruggenmerg, maar er zijn duidelijke verschillen tussen de verschillende (dier)soorten en de test was nog niet gevalideerd bij paarden.

De objectieven van het huidige onderzoek (Hoofdstuk 2) waren dan ook het onderzoeken van TMS-MMEP bij andere diersoorten en het verbeteren en valideren van de techniek bij paarden. De eerste testen bij kalveren werden beschreven in hoofdstuk 3, de verbetering van de techniek in hoofdstukken 4 en 5 en de validatie in hoofdstukken 6 en 7.

In hoofdstuk 3 werd onderzocht of MMEPs ook bruikbaar zijn bij kalveren. Hiervoor werden 41 gezonde kalveren magnetisch gestimuleerd ter hoogte van het foramen magnum. De stimulatie werd uitgevoerd bij staande kalveren en bij kalveren in laterale decubitus. De gemeten latentietijden en amplitudes van de bekomen MMEPs werden gebruikt om referentiewaarden te bekomen in normale kalveren. Bij alle staande kalfjes konden duidelijke en goed reproduceerbare MMEPs geregistreerd worden. Bij de kalveren in laterale decubitus werden er bij slechts 64% van de dieren MMEPs waargenomen in alle 4 de poten. Leeftijd, gewicht, schofthoogte en lichaamstemperatuur hadden geen significant effect op de latentietijd of amplitude en er waren geen significante verschillen tussen links en rechts. De latentietijd was wel duidelijk langer dan in paarden. Dit kan mogelijks verklaard worden doordat de stimulatie ter hoogte van het foramen magnum een ander type respons genereert. Er werd geconcludeerd dat MMEP registratie na magnetische stimulatie ter hoogte van het foramen magnum mogelijk is bij kalveren, maar dat deze moeilijker te bekomen zijn bij kalveren in liggende positie dan bij staande kalveren.

In hoofdstuk 4 werd een eerste aanzet gegeven om de preciezere lokalisatie van ruggenmerg letsels mogelijk te maken in de toekomst. Wanneer chirurgische behandeling van CVM overwogen wordt bij paarden, is correcte lokalisatie van het letsel essentieel voor het succes van de behandeling. Echter, deze correcte identificatie en lokalisatie van het oorzakelijke letsel blijft uitdagend. Bij de mens werd al gebruik gemaakt van MMEPs ter hoogte van de paravertebrale spieren om letsels te lokaliseren, maar dit werd nog niet eerder toegepast bij paarden. Daarom werden door transcraniële magnetische stimulatie motorische potentialen uitgelokt en geregistreerd ter hoogte van de paravertebrale nekspieren bij 50 normale paarden. Ter hoogte van elke nekwervel werden MMEPs geregistreerd om alle individuele cervicaal zenuwen te kunnen evalueren. De nekspier MMEPs konden makkelijk uitgelokt en geregistreerd worden met beperkte variatie tussen de metingen en tussen verschillende waarnemers. De latentietijd werd significant langer van de derde tot de achtste cervicale zenuw, maar ook geslacht en schofthoogte hadden een significante invloed. In de toekomst zouden deze nekspier MMEPs onderzocht moeten worden bij atactische paarden om te bepalen of zij kunnen bijdragen tot de exacte lokalisatie van ruggenmergletsels.

Hoofdstuk 5 beschrijft het gebruik van adhesieve oppervlakte elektroden als alternatief voor de voorheen gebruikte intramusculaire naaldelectroden. De naaldelectroden zijn pijnlijk om te plaatsen en komen vaak los terwijl de oppervlakte elektroden pijnloos geplaatst kunnen worden en stevig blijven zitten. Doch, beide types elektroden meten verschillende dingen. De oppervlakte elektroden meten een samengestelde spier potentiaal die de activiteit van vele motorisch zenuwen weerspiegelt terwijl de naaldelektroden zeer lokaal de MMEPs van enkele spiervezels registreren. Aangezien de referentiewaarden voor TMS-MMEP bij paarden gebaseerd zijn op metingen met naaldelektroden, diende de overeenkomst in latentietijd en amplitude waarden tussen beide types elektroden onderzocht te worden vooraleer kon overgestapt worden naar de oppervlakte metingen. Betreffende de latentietijd werd er een zeer goede overeenkomst vastgesteld tussen naald en oppervlakte elektroden maar niet voor amplitude. Omdat amplitude altijd al een minder betrouwbare parameter was, werd geconcludeerd dat de naald elektroden toch konden vervangen worden door oppervlakte elektroden voor TMS-MMEP registratie bij paarden.

De optimale drempelwaarde voor TMS-MMEP latentietijd voor de diagnose van functiestoornissen ter hoogte van het ruggenmerg werd in hoofdstuk 6 bepaald, gebaseerd op histopathologisch onderzoek bij 5 normale en 17 aangetaste paarden. TMS-MMEP werd eerder al beschreven als een interessante test voor het opsporen van functiestoornissen bij paarden en er is een goede correlatie aangetoond tussen ataxie en verlengde latenctietijden, maar een echte drempelwaarde werd tot nu toe niet bepaald. Door Receiver operating characteristic (ROC)-curve analyse van de latentietijden van de paarden in deze studie, kon de ideale drempelwaarde vastgelegd worden op 22 ms voor de voorbenen en 40ms voor de achterbenen. Indien enkel de gegevens van de paarden met compressie op het ruggenmerg gebruikt werden, steeg de drempelwaarde voor de achterbenen naar 43ms. Met deze drempelwaarden kon een veel hogere sensitiviteit en specificiteit bereikt worden voor de diagnose van ruggenmerg problemen dan tot nu toe beschreven werd voor de halsfoto's. Deze resultaten waren zeer veelbelovend maar moesten nog gevalideerd worden op een groter aantal dieren, waaronder ook dieren met subtiele neurologsiche stoornissen en orthopedische problemen.

De waarde van de in hoofdstuk 6 berekende drempelwaarden werd daarom getest in hoofdstuk 7. De accuraatheid van TMS-MMEP voor de diagnose van functiestoornissen ter hoogte van het ruggenmerg werd berekend door middel van Bayesiaanse statistiek modellen die de resultaten van het neurologisch onderzoek, halsfoto's en TMS-MMEP van 174 paarden combineerden. Op die manier werd een oplossing geboden voor het gebrek aan een gouden standaard voor de diagnose van ruggenmerg functiestoornissen bij levende paarden. De beste test bleek TMS-MMEP, gevolgd door het neurologisch onderzoek. In alle modellen hadden de nekfoto's de laagste accuraatheid voor de diagnose van ruggenmerg stoornissen. Er werden verschillende modellen gebouwd om uit te maken welk beslissingscriterium (minimale of gemiddelde latentietijd van de voor- of de achterbenen) het meest betrouwbaar was. De minimale latentietijd van de achterbenen bleek met een sensitiviteit van 87.5% en een specificiteit van 97.4% het beste diagnostisch criterium.

In de algemene discussie (Hoofdstuk 8) werd geconcludeerd dat TMS-MMEP een eenvoudig uit te voeren test is die goed getolereerd wordt door paarden. Bovendien is de test niet invasief en heeft hij een zeer hoge accuraatheid met vooral een zeer hoge specificiteit. Een diagnostische aanpak voor bevestiging of uitsluiten of ruggenmerg functiestoornissen werd voorgesteld. Bovendien werd het effect van een positief of negatief TMS-MMEP resultaat op de waarschijnlijkheid dat een dier de ziekte heeft, aangetoond door middel van Fagan nomogrammen. Door gebruik te maken van TMS-MMEP zal de diagnose van ruggenmerg stoornissen vereenvoudigd worden en zullen eigenaars meer zekerheid krijgen betreffende de aanwezigheid van de aandoening bij hun paarden. Indien een paard verdacht wordt de aandoening te hebben maar de eigenaar wil graag bevestiging, wordt serieel toepassen van neurologisch onderzoek en TMS-MMEP aangeraden omwille van de zeer hoge specificiteit (99%). Indien ruggenmerg functiestoornissen uitgesloten moeten worden, wordt door middel van parallel testen een zeer hoge sensitiviteit (99.7%) bereikt

CURRICULUM VITAE

Joke Rijckaert werd geboren op 12 februari 1989 te Eeklo. Na het beëindigen van het secundair onderwijs aan "College Onze Lieve Vrouw Ten Doorn" in Eeklo, richting Wiskunde Wetenschappen, startte ze in 2007 met de studies diergeneeskunde aan de Universiteit Gent. In 2013 behaalde ze het diploma van dierenarts (optie paard) met grote onderscheiding.

In oktober van datzelfde jaar ging Joke Rijckaert van start als doctoraat-assistent aan de vakgroep Inwendige ziekten van de grote huisdieren op de faculteit diergeneeskunde in Merelbeke, onder begeleiding van Prof. Dr.P Deprez, Prof Dr. G. van Loon en Dr. B. Pardon en Prof. Dr L. Van Ham van het departement Kleine Huisdieren als specialist neurologie. Naast de algemene dienstverlening in de kliniek en het klinische onderwijs aan en begeleiden van schrijfopdrachten van master studenten, legde ze zich voornamelijk toe op neurologie bij paarden, met de belangrijkste focus op ataxie en het gebruik van transcraniële magnetische stimulatie voor de diagnose ervan.

Joke Rijckaert is auteur of medeauteur van meerdere publicaties in nationale en internationale wetenschappelijke tijdschriften en was meermaals spreker op internationale congressen.

BIBLIOGRAPHY

PUBLICATIONS

Rijckaert, J., Pardon, B., Verryken, K., Van Ham, L., van Loon, G., Deprez, P., 2016. Motor evoked potentials in standing and recumbent calves induced by magnetic stimulation at the foramen magnum. Veterinary Journal 216, 178-182.

Rijckaert, J., Lefère, L., van Loon, G., 2017. Neurologisch onderzoek bij paarden in de praktijk. Vlaams Diergeneeskundig Tijdschrift 86, 47-55

van Galen, G., Saegerman, C., **Rijckaert, J**., Amory, H., Armengou, L., Bezdekova, B., Durie, I., Findshoj Delany, R., Fouche, N., Haley, L., Hewetson, M., van den Hoven, R., Kendall, A., Malalana, F., Muller Cavalleri, J., Picavet, T., Roscher, K., Verwilghen, D., Wehrli Eser, M., Westermann, C., Mair, T., 2017. Retrospective evaluation of 155 adult equids and 21 foals with tetanus in Western, Northern, and Central Europe (2000-2014). Part 1: Description of history and clinical evolution. Journal of Veterinary Emergency and Critical Care (San Antonio) 27, 684-696.

van Galen, G., **Rijckaert, J**., Mair, T., Amory, H., Armengou, L., Bezdekova, B., Durie, I., Findshoj Delany, R., Fouche, N., Haley, L., Hewetson, M., van den Hoven, R., Kendall, A., Malalana, F., Muller Cavalleri, J., Picavet, T., Roscher, K., Verwilghen, D., Westermann, C., Saegerman, C., 2017. Retrospective evaluation of 155 adult equids and 21 foals with tetanus from Western, Northern, and Central Europe (2000-2014). Part 2: Prognostic assessment. Journal of Veterinary Emergency and Critical Care (San Antonio) 27, 697-706.

Rijckaert, J., Pardon, B., Van Ham, L., van Loon, G., Deprez, P., 2018a. Magnetic Motor Evoked Potential Recording in Horses Using Intramuscular Needle Electrodes and Surface Electrodes. Journal of Equine Veterinary Science 68, 101-107.

Rijckaert, J., Pardon, B., Van Ham, L., Joosten, P., van Loon, G., Deprez, P., 2018b. Magnetic motor evoked potentials of cervical muscles in horses. BMC veterinary research 14, 290.

Rijckaert, J., Pardon, B., Saey, V., Raes, E., Van Ham, L., Ducatelle, R., Van Loon, G., Deprez, P. Determination of magnetic motor evoked potential latency time cut-off values for detection of spinal cord dysfunction in horses. Journal of Veterinary Internal Medicine. (Accepted 25/06/2019)

Rijckaert, J., Raes, E., Buczinski, S., Van Ham, L., Deprez, P., van Loon, G., Pardon, B. Accuracy of transcranial magnetic stimulation for diagnosis of spinal cord dysfunction in horses using bayesian latent class modelling. Journal of Veterinary Internal Medicine. (Under review)

ORAL PRESENTATIONS

Paulussen E., Versnaeyen H., **Rijckaert J**., Deneut K., Deprez P., Ducatelle R., Chiers K., van Loon G., 2015. Sidewinder syndrome in a 25 year old welsh cob stallion. European Veterinary Conference Voorjaarsdagen, April 9-11, Amsterdam, Netherlands

Rijckaert J., Lefère L., van Loon G., Deprez P., 2015. Diagnostic aid of transcranial magnetic stimulation in horses suspected of neurological gait abnormalities: a retrospective study. 8th European Congress College of Equine Internal Medicine, November 5-7, Utrecht, Netherlands

Rijckaert J., Pardon B., Verryken K., Van Ham L., van Loon G., Deprez P., 2016. Motor evoked potentials in standing and recumbent calves by magnetic stimulation at the foramen magnum. European Veterinary Conference Voorjaarsdagen, April 13-15, The Hague, Netherlands

Rijckaert J., Lefère L., 2016. Help! Mijn paard heeft zenuwstoornissen. Hoe pak ik data aan? Lecture for the institute of continuing education (IPV), December 14, Merelbeke, Belgium

Rijckaert J., Joosten P., Pardon B., van Loon G., Deprez P., 2017. Magnetic motor evoked potentials of cervical muscles in horses. European veterinary conference voorjaarsdagen, April 19-21, The Hague, Netherlands

Rijckaert J., Pardon B., Saey V., Raes E., Van Ham L., Ducatelle R., van Loon G., Deprez P., 2018. Relationship between ataxia, TMS, cervical radiographs, myelogram and histopathology in 22 horses. 11th European College of Equine Internal Medicine congress, November 7-10, Ghent, Belgium

Rijckaert J., Pardon B., Saey V., Raes E., Van Ham L., Ducatelle R., van Loon G., Deprez P., 2018. Determination of latency time cut-off values for detection of spinal compression-caused neurological dysfunction by transcranial magnetic stimulation in horses. 11th European College of Equine Internal Medicine congress, November 7-10, Ghent, Belgium

Rijckaert J., 2018. Motor evoked potential test : technique and clinical applications. Workshop at the 11th European College of Equine Internal Medicine congress, November 7-10, Ghent, Belgium

Rijckaert J., Pardon B., Raes E., Van Ham L., Buczinski S., Deprez P., van Loon g., 2019. Bayesian comparison of transcranial magnetic stimulation, cervical radiography and clinical examination to diagnose spinal cord dysfunction in horses, European veterinary conference voorjaarsdagen, April 10-12, The Haque, Netherlands

POSTER PRESENTATIONS

Rijckaert J., Van Galen G., Lefère L., De Clercq D., Bauwens C., Broux B., van Loon G., Deprez P., 2015. Tetanus in Equids: 73 cases between 2001 and 2013 at Ghent University. European Veterinary Conference Voorjaarsdagen, April 9-11, Amsterdam, Netherlands

Rijckaert J., Pardon B., van Loon G., Deprez P., 2016. Agreement between needle and surface electrodes for magnetic motor evoked potential recording in horses. 2nd International GEVA-GPM Congress, October 28-29, Berlin, Germany.

Neuckermans Z., **Rijckaert J**., Charalambous M., Bhatti S., Olsen E., Kromhout K., Hoegaerts M., van Loon G, 2019. Case report of complete recovery in an 8-year-old sporthorse with right side forebrain signs from presumptive cerebrovascular origin. European Veterinary Conference Voorjaarsdagen, April 10-12, The Haque, Netherlands

DANKWOORD

Zes jaar geleden was ik super enthousiast dat ik als pas afgestudeerde dierenarts kon starten als doctoraats-assistent bij de vakgroep Inwendige ziekten van de grote huisdieren! Vandaag ben ik nog steeds heel enthousiast, maar vooral ook blij dat dit doctoraat af is. Ik wou graag een doctoraat maken omdat ik vind dat iedereen een stuk(je) verantwoordelijkheid moet nemen om bepaalde problemen op deze wereld op te lossen. Er zijn mensen die oplossingen moeten zoeken om de opwarming van de planeet te stoppen, ik wou graag mijn steentje bijdragen aan de diergeneeskundige vooruitgang. Bij deze vind ik dat ik mijn plicht gedaan heb en terug klinisch werk mag gaan doen. Maar eerst wil ik graag nog een aantal mensen bedanken.

Eerst en vooral, Bart, dank u wel! Zonder u denk ik niet dat dit doctoraat er gekomen was. Proefopzet, sample size berekeningen, statistiek, structuur en inhoud van de papers en bovendien ook mentale ondersteuning. Je hebt me je hulp aangeboden in verband met de kalfjes, en vervolgens kwam ik ook gewoon met mijn volgende onderzoeken bij u aankloppen. Ondanks het feit dat je focus niet bepaald op paarden ligt en dat je een heel “runderteam” (waaronder minstens 4 doctoraatsstudenten, 1 resident en 2 interns) begeleidt, mag het heel duidelijk zijn dat jouw inbreng zeer belangrijk is geweest. Ik vond het een heel aangename samenwerking en ik heb enorm veel van u geleerd. Ik wens je dan ook heel veel succes verder en dat er nog vele publicaties en doctoraten mogen volgen.

Vervolgens wens ik ook professor Deprez te bedanken voor de mooie kansen die ik gekregen heb. Ik weet niet of u het weet, maar u staat ook bekend als “een wandelende encyclopedie”. U weet alles, van de recentste wetenschappelijke literatuur, tot de regels in verband met studenten en doctoraten, van welke informatie je waar kan vinden tot wie de heftruck ’s nachts heeft vastgereden aan de mestput en waar je “Hoe rijd ik met een heftruck” cursussen kunt volgen. Met uw kennis kon u me op tijd in de juiste richting sturen of met de juiste mensen in contact brengen als ik ergens vast zat, maar verder gaf u me vooral ook de vrijheid om mijn eigen onderzoek zelf te ontdekken.

Ook mijn andere promotoren, Professor van Loon en Professor Van Ham wil ik van harte bedanken voor de hulp. Ondanks jullie drukke agenda’s maakten jullie toch tijd om de verschillende artikels en teksten grondig na te lezen en waardevolle suggesties te doen. Mede dankzij jullie heb ik, naar mijn mening althans, toch een behoorlijke evolutie doorgemaakt in het schrijven en logisch opbouwen van wetenschappelijke teksten. Ik kan me best voorstellen

dat jullie bij het eerste artikel toch eens in het haar gekrabd hebben en jullie zich stiekem hebben afgevraagd of dit wel goed zou komen. De volgende artikels warden gelukkig al een stuk vlotter opgebouwd en bij de laatste kreeg ik zelfs commentaren als “zeer goed geschreven” en was het aantal opmerkingen zeer beperkt. Als dat 1 van de doelen is van het schrijven van een doctoraat, denk ik dat jullie missie geslaagd is. Professor van Loon wil ik specifiek ook bedanken voor de logistieke en technische ondersteuning. Als administratief promotor hebt u ervoor gezorgd dat ik hier vandaag voor deze jury en dit publiek kan staan en met uw informatica gerichte suggesties heb ik uiteindelijk ook scherpe figuren in mijn publicaties staan. Het mag gezegd zijn, op informatica gebied heeft u met de dienst inwendige paard, op enkele uitzonderingen na (Glenn), niet het sterkste team rond u, vrees ik. En ik weet niet, zonder namen te noemen, of het er voor de komende jaren veel beter uitziet. Hoe dan ook ziet het er wel naar uit dat er nog vele interessante en baanbrekende cardiologie onderzoeken en doctoraten zullen volgen onder uw begeleiding. Professor Van Ham, de enige échte neuroloog in dit doctoraatsonderzoek, u wil ik persoonlijk bedanken voor het delen van uw kennis. U hebt zelf een doctoraat gemaakt over TMS bij honden en hebt daarna meerdere studies bij kleine huisdieren en paarden begeleidt. U kon dus niet ontbreken. Bedankt ook om me de mogelijkheid te geven enkele dagen mee te lopen bij neurologie kleine huisdieren. Het was interessant te zien dat de problematieken bij kleine huisdieren vaak helemaal anders zijn dan bij paarden, maar dat dezelfde principes wel terug komen. Omgekeerd zijn ook de dierenartsen van de kleine huisdieren neurologie de laatste jaren regelmatig naar de paarden met neurologische klachten komen kijken. Hopelijk kan deze samenwerking tussen de vakgroepen in de toekomst verder gezet worden.

Verder wens ik alle andere leden van de examencommissie te bedanken om mijn werk grondig na te lezen, kritisch te beoordelen en hier vandaag aanwezig te zijn. Professor Goehring, we hebben elkaar de eerste keer ontmoet op het ECEIM congres in Utrecht nadat ik er een korte presentatie gegeven had over de diagnostische waarde van TMS-MMEP. Ik moet eerlijk toegeven dat ik toen nog niet wist wie u was, maar het was wel al een heel aangenaam en interessant gesprek. Intussen heb ik ontdekt dat u veel onderzoek doet en kennis heeft op het vlak van neurologische aandoeningen bij paarden (oa EHV) en histologie van het zenuwstelsel. Bedankt om vanuit München tot hier te komen en deel te willen uitmaken van de jury. Dr. Annick Viane, u maakt deel uit van het revalidatie centrum van UZ Gent en bent dus zeer goed

op de hoogte over hoe beweging werkt en wat er allemaal kan misgaan in geval van neurologische ziekteprocessen. Ondanks het feit dat u normaal alleen met mensen werkt, was u zeer geïnteresseerd in deze studie bij paarden en meteen enthousiast om deel uit te maken van deze jury. Bedankt daarvoor en heel veel succes met uw eigen verdediging binnenkort. Dr Dumoulin, Michèle, bedankt om in mijn jury te zetelen maar ook voor de samenwerking de voorbije jaren. Als orthopedist zie je regelmatig een neurologisch abnormaal paard passeren en als internist sturen we regelmatig een paard naar orthopedie. Ik hoop dat TMS-MMEP ook naar de toekomst toe een meerwaarde voor jullie kan betekenen. Dr Bhatti, Sophie, ook u wil ik bedanken om deel uit te maken van de jury en voor de samenwerking. Uw focus ligt natuurlijk bij de kleine huisdieren, maar toch is het altijd interessant uw visie te horen als we een paard hebben met atypische neurologische klachten. Professor Dewulf, bedankt om voorzitter te willen zijn en de verdediging in goede banen te leiden.

Bedankt ook aan alle collega's van Medische beeldvorming voor het nemen van alle halsfoto's en echo's. Voor het sleuren aan de paarden bij de myelogrammen en CT scans. Bedankt voor het interpreteren van de beelden en het uitrekenen van inter- en intravertebrale ratio's. Elke, bedankt (en sorry) voor de CT scans op verlofdagen. En vooral Els, heel erg bedankt voor het blind herbekijken en interpreteren van de halsfoto's van de 174 paarden voor mijn 2 laatste studies. Ik besef dat dat ongelooflijk veel werk is geweest!

Als er over "veel werk" gesproken wordt, moet ik ook de dienst pathologie uitvoerig bedanken. In de eerste plaats Dr. Veronique Saey voor het beoordelen van alle histologische coupes en Professor Ducatelle voor de hulp bij de interpretatie van de resultaten. Maar natuurlijk moet ik ook iedereen bedanken die de lijkschouwingen en het histologisch onderzoek mogelijk maakt, van technisch personeel tot residents, van studenten tot diplomates en professoren. Bedankt voor al jullie hulp en het bijhouden en verwerken van de stalen.

Ook de collega's van heelkunde en anesthesie wil ik bedanken voor de vlotte samenwerking met betrekking tot het klinisch beoordeling van patiënten door de orthopedisten, de anesthesie tijdens de onderzoeken en de vlotte planning ervan, het overleg met de chirurgen in verband met therapeutische opties en de mensen van het secretariaat om alles goed te plannen. Het was een aangename samenwerking, ik heb veel van jullie geleerd.

Verder wil ik graag alle dierenartsen bedanken die paarden doorgestuurd hebben vanuit België, Nederland, Frankrijk, Duitsland,… voor een TMS-MMEP onderzoek en alle eigenaars die tot hier gekomen zijn. Keros en de vakgroepen heelkunde en verloskunde wil ik bedanken voor het tijdelijk uitlenen van hun paarden voor mijn proeven. Ook zonder jullie was dit doctoraat niet mogelijk geweest.

Dan kom ik stilaan bij mijn eigen vakgroep terecht, inwendige ziekten van de grote huisdieren. Ik heb hier de voorbije jaren zeer graag gewerkt. Enerzijds uit interesse voor de inwendige ziekten maar zeker ook dankzij de fantastische groep collega's. Sabrina, Sylvie, Elvin, Hans, Saar, Tony, Franky, Balder, Carlos en Julien, bedankt voor al jullie administratieve en technische ondersteuning en de babbels uit het leven. Bedankt aan "het runderteam" Bonnie, Linde, Lieze, Laura, Kat, Jade, Mathilde en Charlotte om jullie te ontfermen over de TRP koeien, diarree en pneumonie kalveren, alpaca's met huidproblemen, eenzame varkens, schapen die meubels eten, geiten met endometritis en hun eigenaars, aanvallende kamelen en wallaby's,… Jullie zijn goed bezig! Laura, Kat en Jade, succes met jullie doctoraten! Samen helpen jullie pneumonie de wereld uit. Lieze, heel veel succes met het verdere verloop van je residency.

Dominique, Glenn en Lisse, bedankt voor alle suggesties in de proefpresentaties voor de verschillende congressen waar we naartoe mochten gaan. Universiteit Gent was vaak goed vertegenwoordigd op de abstract presentaties en ging bovendien ook geregeld met de prijzen lopen dankzij jullie spectaculaire onderzoeken. Jullie zijn goed bezig en vormen een heel sterk onderzoeksteam. Heel veel succes met de doctorate!

Annelies, met Valentine, Alix, Pia en An heb je een heel leuk en bekwaam skillslab en communicatie team verzameld. Ik hoor alleen maar positieve reacties van studenten. Heel veel succes met de verdere uitbouw hiervan. Valentine, bedankt om dit doctoraat na te lezen en bedankt voor het meedenken over de patiënten bij het koffie apparaat of tijdens het eten. Pia, veel succes met je doctoraat.

An, jij zit goed bij deze vakgroep want "you have skills" én je kan uitstekend communiceren. Op dat laatste hebben we hebben dan ook al véél geoefend de laatste jaren. In 2013 zijn we samen Caroline begonnen op inwendige. Dat is een heel gezellig jaar geworden waar ik eigenlijk alleen maar goede herinneringen aan overhoudt. Daarna heb je ons met veel drama (cava, tranen en karaoke) verlaten omdat je de wereld wou zien. Je bent tot in Frankrijk geraakt waar je uiteindelijk toch besefte dat je ons niet kon missen en teruggekeerd bent. Ik

was alleszins heel blij je terug te zien, het leek alsof je nooit was weggeweest, het enige verschil was dat je nu je super enthousiaste en levendige hond Ruby meebracht. Intussen is er wel het een en het ander veranderd en zijn er grote stappen genomen naar volwassenheid. Bedankt voor de mooie jaren en veel succes met alles wat nog komt, maar ik ben zeker dat wij elkaar nog vaak gaan zien.

Dat brengt me naadloos naar de collega's van kliniek inwendige paard. Jullie zijn heel leuke collega's om mee samen te werken (als we er allemaal aan denken om lief te zijn voor elkaar)! Maar jullie zijn vooral ook veel meer dan dat. Op werk vlak was alles redelijk stabiel maar op persoonlijk vlak zijn de voorbije jaren, voor mij toch, op zijn minst wat "rumoerig" geweest. Gelukkig heb ik van jullie heel veel "steun" gehad. Ik weet niet of het altijd steun genoemd kon worden, maar laat ons zeggen dat jullie me de humor van de situatie konden laten inzien. Verder werden er vooral ook veel cava'tjes gedronken samen en veel feestjes gevierd. Er werden veel (te veel) persoonlijke details uitgewisseld en er werd samen naar de sterren gekeken op zoek naar de grote beer. We hebben er toch het beste van gemaakt! Laurence, Caroline, Barbara, Ellen, Alex, Zoé, Lisa. Bedankt voor alles.

Laurence, la mamma, de moederkloek van inwendige, ik heb ongelooflijk veel respect voor wat jij doet en voor de dierenarts die je bent. Jij bent de vaste waarde voor de kliniek, de (jonge) dierenartsen en de studenten. Ondanks je jarenlange ervaring en zin voor realiteit blijf je openstaan voor verandering en nieuwe therapieën.

Caroline, wij hebben veel diensten samen gedaan. Maar je was precies niet altijd even enthousiast als ik je belde dat er een koliek op komst was, en nog een… en nog een. De wandeling met de paarden die jij georganiseerd had was een heel memorabele!

Barbara, intussen ben je diplomate, doctor, mama en heb je andere oorden opgezocht maar toen ik hier startte was je pas assistent. Om maar te verduidelijken dat je niet hebt stilgezeten. Ik heb vele leuke herinneringen aan onze tijd op het werk en er naast, van "ik moet NU een koekje hebben" tot "oeps, nu weet je het geslacht van de baby". Heel veel succes daar in Frankrijk!

Ellen, ook jij bent in 2013 gestart aan de faculteit. Je zat weliswaar nog even op een dwaalspoor maar na een jaartje heb je toch de juiste weg gevonden. Nu ben je diplomate en ben je ook aan een doctoraat gestart. Goed bezig! In tussentijd hebben we heel wat

afgelachen. Onze privélevens hebben ietwat gelijkaardige wendingen gekend in dezelfde periode waardoor er heel veel interessante verhalen en ervaringen konden worden uitgewisseld. Als ik eraan terug denk sta ik nog versteld van wat jij allemaal hebt uitgespookt! Gelukkig is het voor ons beiden uiteindelijk toch allemaal goed gekomen. Heel veel succes met doctoraat en de bouw van je droomhuis.

Alex, ooit was ik degene van wie de studenten (en interns) schrik hadden, mogelijks heb jij intussen die rol overgenomen. Jij test graag de kennis van studenten maar wordt helaas vaak teleurgesteld, mede omdat jouw kennis enorm uitgebreid is geworden. En als daar ook nog eens een doctoraat bijkomt, zal het er niet op verbeteren. Je bent ongelooflijk gemotiveerd en hebt een hele evolutie doorgemaakt. Ik wens je heel veel succes in je verdere carrière, nog veel Helene Fischer concerten en een ezel.

Zoé en Lisa, onze junior residents. Jullie hebben beiden veel potentieel. Jullie komen er wel! Jullie zijn in een goed team terecht gekomen. Heel veel succes met jullie opleiding!

Ook een dikke merci aan alle interns van de voorbije jaren, Alex, Jasmien, Helen, Zoé, Phyllis, Gerry, Lisa, Anke, Hélène, Céline, Sylvie en Paulien, voor jullie harde werk, het soms riskeren van jullie levens en het aangenaam gezelschap.

Gelukkig was er ook nog tijd voor een leven naast het doctoraat waarin heel wat vaste waardes voorkomen. Valerie, Willem, Jeroen, Heleen, Elien, Stefaan, Ellen, Sofie, Delphine, paardrijvrienden,… bedankt voor de fietstochtjes, etentjes, uitstapjes en berichtjes. Het doet deugd te weten dat jullie er zijn! Bedankt voor jullie aanwezigheid vandaag. Ik apprecieer het heel erg!

Anouk, hoeveel uren zouden wij al niet samen op pad geweest zijn, paardgereden en jenevers of gin tonics gedronken hebben. Ondanks het feit dat ik zo ver gaan wonen ben, zien en horen we elkaar heel regelmatig. Ik ben heel blij dat ik Ruíz zijn meter mocht worden. Dat moet echte vriendschap zijn! Merci!

Ook bedankt aan mijn familie, nichtjes, neven, tantekes, nonkels, oma voor de steun en de interesse. Jullie zijn echt een toffe familie! Opa, meme en pepe zouden vast trots geweest zijn. Ook bedankt aan heel de familie van den Bosch om mij op te nemen in de familie.

Jonas, je bent een fantastische broer. Jij steunt me door dik en dun. Je kan alles. Samen met Kim, Kytano, Kobe en mijn metekindje Jarne vorm je een prachtig gezinnetje. Zorg goed voor elkaar!

Mama en papa, ik vermoed dat jullie wat betreft het doctoraat niet altijd goed konden volgen maar jullie waren wel gewoon trots. Trots op mijn werk als dierenarts, dat ik lessen mocht geven, artikels publiceerde en naar internationale congressen ging. Ook jullie staan 100% achter mij en ik kan altijd bij jullie terecht. Voor een overnachting met ontbijt maar vooral ook met al mijn verhalen, problemen, plannen en vragen. Ik weet niet hoe jullie het doen, maar ik wordt er altijd wijzer van, al geven jullie zelden kant en klare oplossingen. Jullie hebben me geleerd dat problemen er zijn om opgelost te worden. Een gegeven dat zeer interessant was in het kader van dit doctoraat maar vanzelfsprekend ook in het dagelijkse leven. Dank jullie wel!

Lawrence, jij bent mijn grootste supporter maar tegelijk ook mijn grote voorbeeld. Ik heb enorm veel bewondering voor wie je bent als persoon en wat je verwezenlijkt. Dankzij jou geloof ik dat alles mogelijk is en dat dromen werkelijkheid kunnen worden.

Dank u!