



FACULTY OF VETERINARY MEDICINE
approved by EAEVE

Bovine spastic paresis: diagnosis and treatment of atypical presentations

Caroline De Vlaminck

Dissertation submitted in fulfillment of the requirements
for the degree of Doctor of Philosophy (PhD) in Veterinary Sciences

2015

Promoters:

Prof. Dr. L. Vlaminck

Prof. Dr. F. Pille

Dr. I. Van Soens

Department of Surgery and Anaesthesiology of Domestic Animals

Faculty of Veterinary Medicine

Ghent University

Printed by Reproduct, Ghent, Belgium.

www.reproduct.be

Bovine Spastic Paresis: diagnosis and treatment of atypical presentations

Caroline De Vlamynck

Department of Surgery and Anaesthesiology of Domestic Animals

Faculty of Veterinary Medicine

Ghent University

ISBN:

TABLE OF CONTENTS

LIST OF ABBREVIATIONS	5
PREFACE	9
CHAPTER 1	11
Introduction.....	
Bovine spastic paresis: an overview	
CHAPTER 2.....	37
Scientific aims.....	
CHAPTER 3.....	41
Long-term outcome of conservative management or surgical treatment of bovine spastic paresis: 79 cases	
CHAPTER 4.....	61
Diagnosing quadriceps femoris muscle involvement in bovine spastic paresis.....	
SECTION 4.1	63
Femoral nerve blocking: cadaver study.....	
SECTION 4.2	83
Femoral nerve blocking: clinical trial	
CHAPTER 5.....	103
Surgical management of atypical presentations of bovine spastic paresis.....	
SECTION 5.1	105
Recording of cord dorsum potentials in calves	
SECTION 5.2	119
Selective dorsal rhizotomy to tackle quadriceps femoris muscle spasticity in cattle: a pilot study.....	

CHAPTER 6	145
General discussion	
SUMMARY.....	165
SAMENVATTING	171
CURRICULUM VITAE.....	177
BIBLIOGRAPHY	179
DANKWOORD.....	181

LIST OF ABBREVIATIONS

BSP	Bovine Spastic Paresis
BSP-G	Bovine Spastic Paresis of the gastrocnemius muscle
BSP-M	Mixed presentation of Bovine Spastic Paresis
BSP-Q	Bovine Spastic Paresis of the quadriceps femoris muscle
Ca	Caudal vertebra
CDP	Cord Dorsum Potential
EMG	Electromyography
IV	Intravenous
L	Lumbar vertebra
OL	Onset latency
PL	Peak latency
PPA	Peak-to-peak amplitude
SD	Standard deviation

*'Keep your face to the sunshine
and you cannot see a shadow'*

Helen Keller

PREFACE

Bovine spastic paresis is a well-known progressive neuromuscular disease in cattle. Economic losses are due to decreased weight gain and early slaughter of affected animals. Original reports discuss bovine spastic paresis of the gastrocnemius muscle (BSP-G) in which the affected hind limb is typically stretched in a caudal direction. Successful surgical treatment options are known for this type of spastic paresis. More recently, spasticity of other muscle groups has been reported. Spastic contractions of the quadriceps femoris muscle group (BSP-Q) result in a cranially directed pendulous movement of the affected hind limb. In mixed presentations, spasticity of the gastrocnemius and/or the quadriceps femoris muscle and/or potentially other muscle groups of the hind limb was observed (BSP-M) causing cranially, caudally or laterally directed movements of the affected hind limb depending on the dominant spastic muscle group. There is no objective diagnostic tool available to differentiate between the three entities. Moreover, for the BSP-Q and BSP-M animals, no specific therapy exists nor have there been reports published to document the evolution of the clinical signs and outcome.

Hence, this PhD work focuses on the diagnosis and management of atypical cases of spastic paresis.

CHAPTER 1

Introduction

Bovine spastic paresis: an overview

Adapted from:

De Vlaminck C., Pille F., Vlaminck L., 2014. Bovine Spastic Paresis: Current Knowledge and Scientific Voids. *The Veterinary Journal* 202, 229-235.

Introduction

Bovine spastic paresis (BSP) is a progressive neuromuscular disease characterised by spastic contractions of one or more muscles of the hindquarters and/or back in standing cattle. With the exception of some breeds, particularly the Belgian Blue and Romagnola, the gastrocnemius muscle is most commonly affected, with spastic paresis causing the animal to repetitively stretch the affected limb in a caudal direction.

BSP was first described by the Professor Joseph Hamoir in 1922 and reported to be a heritable disease originating from the East Friesian bull Elso II (Götze, 1932). Götze (1932) introduced the term 'spastic paresis' to describe the abnormal muscular contractility. Subsequently, the condition has been reported in many breeds of cattle throughout the world. Spastic paresis has also been observed, although infrequently, in goats (Baker et al., 1989).

The physical condition of affected animals deteriorates rapidly, owing to the constant pain and stress evoked by the muscle spasms. This makes effective treatment essential. The first proposed treatment was tenotomy of the spastic muscles (Götze, 1932). This was later modified to avoid hyperflexion of the tarsocrural joint (Pavaux et al., 1985). De Moor et al. (1964) introduced selective neurectomy of the branches of the tibial nerve supplying the gastrocnemius muscles, based on a technique for denervating the gastrocnemius muscle in human beings. Bijleveld (1973) proposed the use of a total tibial neurectomy.

Abnormalities of muscle groups other than the gastrocnemius may be implicated in spastic paresis. Denniston et al. (1968) were the first to show the involvement of multiple muscle groups; this has now been recorded in several breeds of cattle and is generally referred to as the 'mixed presentation of bovine spastic paresis' (Vertenten, 2006). Spastic paresis, in which only the quadriceps femoris muscle is involved, has been described in Belgian Blue (Touati et al., 2003) and Romagnola cattle (Gentile et al., 2006). To date no suitable surgical treatment exists for animals with spastic paresis of muscles other than the gastrocnemius.

Introduction

Although research has shed some light on the aetiopathogenesis of BSP, much remains to be elucidated (De Ley and De Moor, 1975; De Ley and De Moor, 1977; De Ley and De Moor, 1980; Pariset et al., 2013). In this chapter, the current knowledge of the condition is reviewed and areas of uncertainty are identified.

Clinical presentation

Bovine spastic paresis is most commonly associated with repetitive contractions of the gastrocnemius muscle although the symptoms can originate from other muscle groups such as the quadriceps femoris muscle or from a combination of hind limb muscles (Touati et al., 2003). A recumbent animal usually will show no overt clinical symptoms, but spastic contractions become visible as soon as it rises or attempts to stand up (Keith, 1981; Harper, 1993; Touati et al., 2003; Vertenten, 2006). The age at which clinical signs become apparent ranges from 1 day up to 3 years (Vlaminck et al., 2000; Touati et al., 2003). Initially, clinical signs are subtle, but they progressively worsen over time. Involvement of both unilateral and bilateral hind limbs is possible (Touati et al., 2003).

Bovine spastic paresis of the gastrocnemius muscle

The gastrocnemius muscle is a flexor of the stifle and the main extensor of the hock (Figure 1). It is innervated by the tibial nerve (Barone, 2000).

Bovine spastic paresis of the gastrocnemius muscle (BSP-G) is characterised by an increased tibiotarsal angle and a hind limb that is stretched caudally (Figure 2 top). The back is frequently arched and the tail head can be elevated (Keith, 1981; Harper, 1993). When affected unilaterally, the calf will stand on the unaffected limb while the toe of the extended contralateral limb will not touch the ground. Walking is difficult as the affected limb is spastically drawn caudally with each step. When affected bilaterally, the animal will show marked extension of both hind limbs and continuous weight shifting. Upon palpation, increased tone of the gastrocnemius muscle and its tendon may be detected. As the disease progresses and severity of the symptoms increases, affected cattle become reluctant to stand up. Chronic cases develop gluteobiceps muscle atrophy and remodelling of the calcaneus which tends to incline towards the tibia due to the repetitive strain (Keith, 1981). The epiphysis of the calcaneus may be enlarged and irregular (Frederik and Van 't Hooft, 1962).

Bovine spastic paresis of the quadriceps femoris muscle

The quadriceps femoris muscle is a large muscle group that includes the four prevailing muscles on the front and side of the femur. It is the major extensor muscle of the stifle (Barone, 2000). The quadriceps femoris muscle is innervated by the femoral nerve (Figure 1).

BSP of the quadriceps femoris muscle is usually bilateral (Touati et al., 2003). While standing, the animal is weight shifting with a convexely arched back (kyphosis) while the non-weight bearing hind limb is stretched cranially (Figure 2 middle). Upon palpation, increased quadriceps femoris muscle tonus is detectable. When walking, the hind limbs are rigidly advanced with a swinging pendulum motion, resembling the gait of a tin soldier (Touati et al., 2003). When affected unilaterally, the calf will stand on the unaffected limb with the spastic limb stretched cranially. Bovine spastic paresis of the quadriceps femoris muscle (BSP-Q) has been described in Romagnola cattle and Belgian Blue calves (Touati et al., 2003; Gentile and Testoni, 2006).

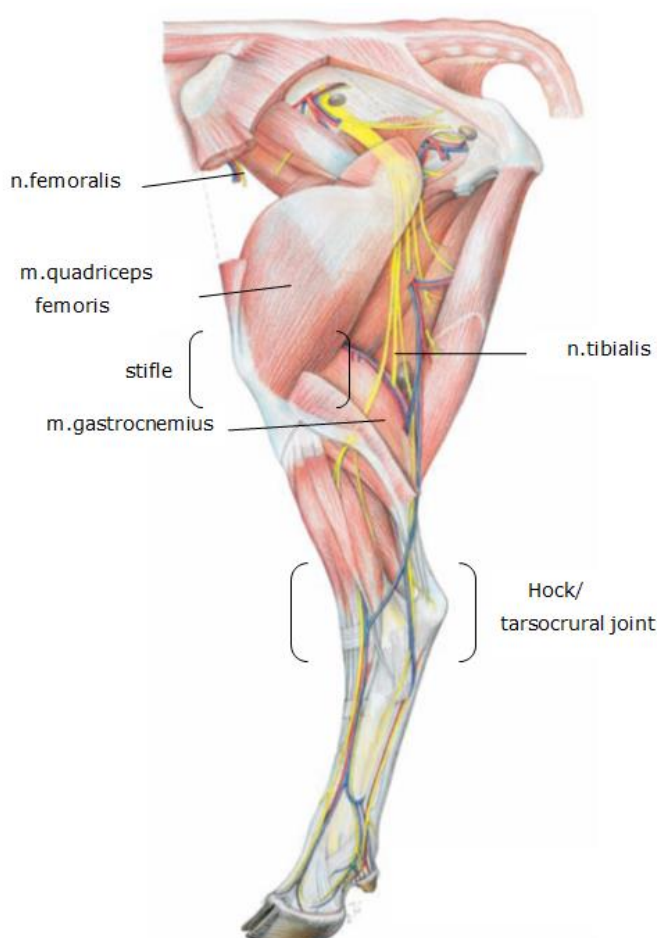


Figure 1 - Lateral thigh and cranial crural muscles with their nerves. (Adapted from Budras and Habel, 2003)

The m. tensor fasciae latae, m. gluteus medius, and m. biceps femoris (m.gluteobiceps) are severed at their origin and insertion and removed.

Mixed presentation of bovine spastic paresis

In recent years, a higher frequency of involvement of multiple muscle groups in bovine spastic paresis cases (BSP-M) has been observed in Belgian Blue calves referred to our large animal clinic (Vertenten, 2006). Spasticity of gastrocnemius, quadriceps femoris and probably other muscle groups of the hind limb cause a swaying hyperextension of the tibiotarsal joint in alternating and varying cranial, caudal and even lateral directions in the standing animal (Figure 2 bottom). Based on clinical signs, identification of involved muscle groups is challenging especially in the early stages of the disease.



Figure 2

Top: Zebu suffering from bovine spastic paresis of the gastrocnemius muscle (BSP-G). The left hind limb is stretched in caudal direction.

Middle: Calf suffering from bovine spastic paresis of the quadriceps femoris muscle. Both hind limbs are spastically stretched in cranial direction, resulting in a convexly arched position of the back (kyphosis).

Bottom: Extremely muscled calf suffering from the mixed presentation of bovine spastic paresis. In this case, the left hind limb is stretched laterally.

Epidemiology

Spastic paresis was first reported in Friesian cattle in Germany, but since then it has been reported in many cattle breeds, including Belgian Blue, Holstein Friesians, Charolais, Romagnola, Brahman crossbreeds, Herefords, Beef Shorthorn, Jersey, Aberdeen Angus and Ayrshire cattle, Meuse-Rhine-Yssel, Groninger, Brown Swiss, Red Danish, Hungarian and Czech Red Spotted, Gelbvieh, Japanese black and Krankrej (Götze, 1932; Formston and Jones, 1956; Wheat, 1960; van Gastel-Jansen and Frederik, 1962; Love and Weaver, 1963; Rasbech, 1963; Roberts, 1965; Bouckaert and De Moor, 1966; Leipold et al., 1967; Denniston et al., 1968; Gadgil et al., 1970; Arnault, 1982; Browning et al., 1986; Thomason and Beeman, 1987; Harper, 1993; Vlaminck et al., 2000; Gentile et al., 2002; Miura et al., 2009).

Even though there is a clear genetic link to the disease, to the best of our knowledge no cattle breed societies have kept or keep records of the prevalence of spastic paresis. Reports suggest that the prevalence is < 1 %; for example, Gentile et al. (2002) reported a prevalence of 0.8% in Romagnola cattle, while Ledoux (2001) stated that the prevalence in French cattle was 0.1% and declining. However, without objective epidemiological data these figures cannot be confirmed. Such data are needed across breeds and countries to better establish the impact of the condition.

Since BSP was first reported, it has been clear that there is a hereditary component to the disease, with the initially affected animals being traceable to a single Friesian sire, Elso II (Götze, 1932). Other investigations have also shown a clear genetic link; in affected Polish cattle, Szteyn et al. (1972) showed a link with the genetic line from Annas Adema. Nevertheless, the mode of inheritance and the penetrance of the responsible genes have not been elucidated. Additionally, although the disease is more frequently observed in male animals, no clear sex predilection has been proven (Arnault, 1982; Vlaminck et al., 2000; Griffiths, 2005).

Pathogenesis

It is believed that an overactive stretch reflex is responsible for the symptoms of bovine spastic paresis. Elongation of the stretch-sensitive muscle spindles, located parallel with and between the extrafusal muscle fibers, results in an impulse, carried to the spinal cord by the Ia nerve fibers of the dorsal root, which monosynaptically excites the alpha motor neurons in the ventral horn of the spinal cord. This signal reaches the motor end-plates of extrafusal muscle fibers, resulting in an increased muscle tone (Figure 3). Gamma motor neurons located in the ventral horn of the spinal cord influence the stretch sensitivity of the muscle spindles. Impulses, arising either from the brain, the spinal cord or locally, determine the inhibitory or excitatory state of the gamma motor neurons. The hypothesis of an overactive stretch reflex in calves affected with spastic paresis was shown in three calves by disappearance of symptoms after selective resection of dorsal spinal roots of the tibial nerve at the level of the L4, L5 and L6 vertebrae (De Ley and De Moor, 1977). Hence, the motor fibers in the ventral nerve roots remained intact while the sensory fibers of the dorsal roots were resected in order to eliminate the stretch reflex without denervation of the muscle. In another study, spastic paresis symptoms could be masked after selective suppression of gamma motor neurons within the tibial nerve by epidural administering of a 0.38 per cent procaine solution (De Ley and De Moor, 1980). This concentration of procaine only blocks the thinner gamma motor neurons while leaving nerve fibers of larger diameter (alpha motor and Ia sensory neurons) functionally intact. It was concluded that spastic contractions of the gastrocnemius muscles might be caused by overstimulation and/or lack of inhibition of gamma motor neurons.

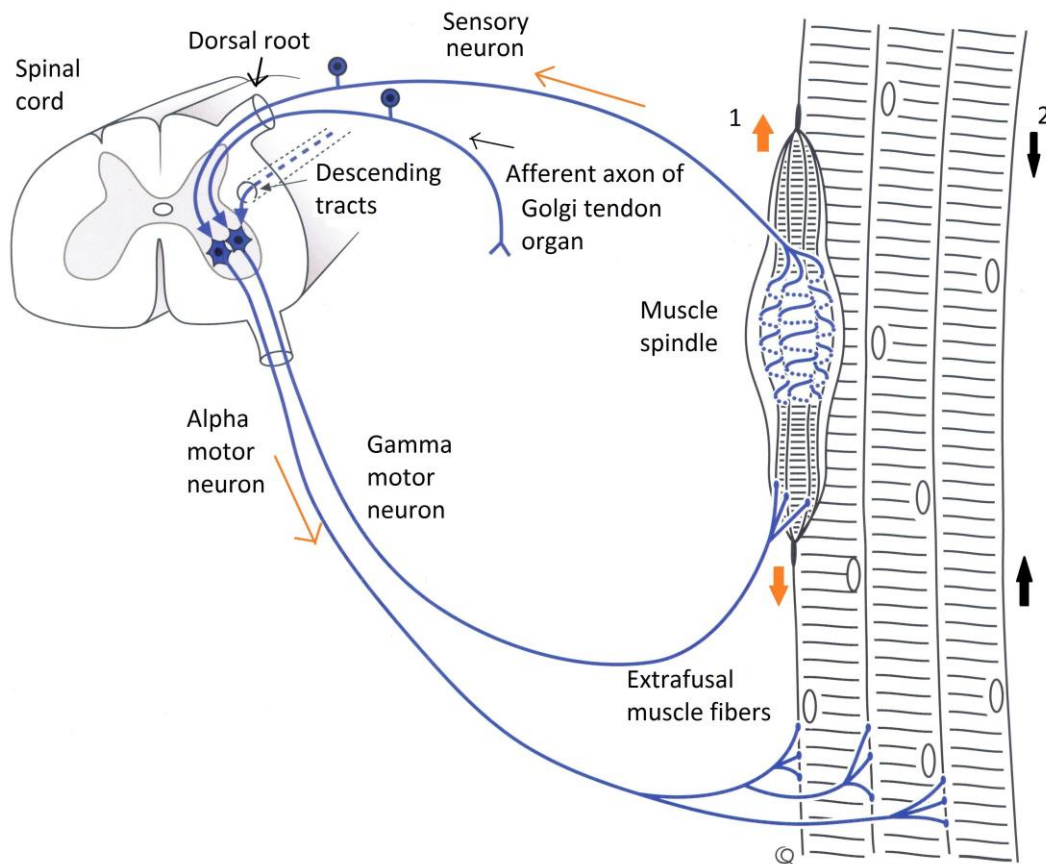


Figure 3 - A simplified drawing of the gamma motor neuron system (adapted from Mayhew, 2009).

During the stretch reflex, the extrafusal muscle fibers of the muscle are initially lengthened and as a consequence the muscle spindles are also stretched (1). As a result, a signal is sent through sensory neurons (Ia motor neurons) towards the spinal cord via the dorsal root. The signal is monosynaptically directed to the alpha motor neurons which results in muscle contraction, pulling back the extent of the stretch (2). Gamma motor neurons, located in the ventral horn of the spinal cord, regulate the sensitivity of the stretch reflex by tightening or relaxing the fibers within the muscle spindle. These gamma motor neurons are controlled by descending upper motor neuronal pathways and by local pathways e.g. proprioceptive input from the Golgi tendon organs. In animals suffering from bovine spastic paresis, an overstimulation and/or lack of inhibition of gamma motor neurons is supposed.

Whether this neurologic deficit takes place at a central, spinal or peripheral level remains unclear. Central nervous system dysfunctions have been proposed. The concentration of a dopamine metabolite, homovanillic acid, was found to be lower in the cerebrospinal fluid of affected calves, indicating that a lower dopaminergic metabolism takes place in the central nervous system of spastic paresis affected animals (De Ley and De Moor, 1975). However, an immunohistologic study of different areas of the brain in normal and spastic calves could not reveal significant differences of dopamine concentrations (Dewulf et al., 1982).

Based on lithium and tryptophan treatment trials, Arnault (1979) suggested an anomaly at the synaptic level of the serotonergic system, which has an important function in the red nucleus. However, the concentration of 5-hydroxyindoleacetic acid, the main metabolite of serotonin, was within normal values in cerebrospinal fluid of calves affected with bovine spastic paresis (De Ley and De Moor, 1975). This result may be explained by the fact that a partial deficiency of the serotonergic systems in the brain would have little impact on 5-hydroxyindoleacetic concentrations in cerebrospinal fluid (Ledoux, 2001).

Chiocchetti et al. (2006) suggested that the overactive stretch reflex resulted from a lack of inhibitory mechanisms in the central nervous system. In their study, the average cross-sectional area of neurons in the red nucleus and the medullary reticular formation was found to be significantly lower in affected calves than in normal calves. These two neuronal centers have an inhibitory action on the lower motor neurons which control limb extension.

In a recent genetic study, tests were performed on spinal cords of healthy and spastic calves to identify metabolic pathways associated with the disease. The results suggested that the development of the disease is linked to a defective glycinergic synaptic transmission and an alteration of calcium signaling proteins (Pariset et al., 2013). Glycine is one of the most important inhibitory neurotransmitters in the spinal cord, and glycinergic synapses have a well-established role in the regulation of locomotor behavior (Legendre, 2001). In neurons, intracellular calcium signals have crucial roles in activating neurotransmitter release and in triggering alterations in neuronal function.

Introduction

Calcium signaling proteins can bind free calcium in the cytosol, influencing the intracellular signals (Burgoyne, 2007).

Histopathological studies have demonstrated degenerative lesions in the central nervous system associated with spasticity of the gastrocnemius. Vacuolated nerve cells in the red nucleus and gliosis in both grey and white matter in various parts of the central nervous system have been reported in young calves with spastic paresis. However, these findings were not consistently reported in all affected animals, and were occasionally observed in unaffected calves (Chomiak and Szteyn, 1970; Milart and Chomiak, 1971; Chomiak et al., 1972; Baird et al., 1974; Dewulf et al., 1982). In a 3-year-old bull, spastic paresis was associated with signs of cellular degeneration, mainly vacuolation and tigrolysis in the extrapyramidal neurological system which contains the principal centres of control and regulation of motor functions (Lewandowski et al., 1970). These changes were also observed, in a mild form, in a 13-year-old bull free from clinical signs, but which had sired four bulls with spastic paresis (Szteyn et al., 1972). Neuronal vacuolation has more recently been identified as a non-specific lesion seen in healthy older cattle in the red nucleus and oculomotor nucleus region (Ledoux, 2001). Thus, the degenerative lesions that have been reported in the central nervous system of cattle with spastic paresis do not appear to be characteristic of or consistent with BSP.

Histopathological examination of the spinal cord and tibial nerves of affected animals could not reveal any abnormalities (Denniston et al., 1968; Chomiak and Szteyn, 1970; Baird et al., 1974). Histopathological studies of muscle tissue are scarce but no primary histological lesions have been reported in the spastically-affected gastrocnemius muscle (Love and Weaver, 1963; Leipold et al., 1967). One study found eosinophilic vasculitis, which disappeared after tibial neurectomy, and was believed to be the result of irritation due to the repetitive muscle contractions (Denniston et al., 1968).

Aetiology

The aetiology of spastic paresis remains unknown. Since clinical signs mostly develop several days to weeks following birth, spastic paresis is classified as a developmental disease. Breeding experiments suggest a genetic model including both autosomal and recessive genes with incomplete penetrance (Bijleveld and Binkhorst, 1973; Van Huffel et al., 1986; Hanset et al., 1993). It is likely that some calves have an inherited predisposition to spastic paresis which only becomes clinically observable in the presence of certain unidentified environmental, nutritional, metabolic or other conditions (Dawson, 1975; Van Huffel et al., 1986).

In the crooked tail syndrome, which is a well known hereditary disease in the Belgian Blue breed, BSP-Q and BSP-M is regularly observed as an additional genetic disorder (Fasquelle et al., 2009). In this syndrome, calves mainly display tail deviation, general growth retardation, a short, broad head and an extreme muscular hypertrophy. Spastic paresis of the quadriceps femoris muscle only is observed in 22 % of calves suffering from the crooked tail syndrome, spastic paresis of the gastrocnemius and quadriceps femoris muscle together is observed in 14% of the cases (Fasquelle et al., 2009).

Microarray analysis of the spinal cords of normal and spastic Romagnola calves has found 268 genes that are significantly over/under expressed. Further analysis based on pathway mapping revealed that the over/under expressed genes were predominantly involved in cell communication, signalling molecules and their interaction, signal transduction and the nervous system (Pariset et al., 2013).

Nutritional deficiency has also been suspected as a factor in the development of spastic paresis. Arnault (1979) evaluated the effect of administering 2 g/100 kg bodyweight tryptophan intramuscularly for 2 to 3 days and found that it was effective in 7/9 calves with limited symptoms of spastic paresis (i.e. hyperextension of the tarsocrural joint). Treatment of calves with more severe symptoms was unsuccessful.

Introduction

Similarly lithium supplementation (4 g/100 kg) was effective in 50/65 Charolais calves in the first days of the disease, but not in calves with more severe symptoms (Arnault, 1979). In successful cases, aggravation of the symptoms was often noted during the first 5 days of treatment. Arnault (1979) also reported that combined administration of manganese, copper and lithium were more effective than lithium treatment alone, but this effect was not proven statistically. The same author (Arnault, 1979; Arnault, 1982) also reported a positive outcome of copper supplementation in calves with a subclinical presentation of spastic paresis. Calves showing spastic contractions of the hind limbs did not respond to this treatment. The subclinical phase was defined as the appearance of a straight hock, with a stiff, inharmonious, mechanical type gait, without rhythmic spastic contractions of the hind limbs. However, the method by which the hock was classified as 'straight' and how a physiologically normal straight hock was distinguished from a pre-spastic tibiotarsal joint were not described. Moreover, the criteria used to evaluate treatment success in these studies were not provided. Even if these studies do reflect the true response it is likely that lithium and tryptophan (in particular) do not act by correcting a general deficiency, but by restoring the operation of a group of neurones (Ledoux, 2001).

De Ley and De Moor (1975) suggested that manganese deficiency was associated with hyperextension of the tibiotarsal joints, but studies of induced manganese deficiency in identical twin calves have found no effect on the angle of the tarsocrural joint (Van Huffel et al., 1986).

Correlations between the occurrence of spastic paresis and environmental factors such as season of birth (Stegenga, 1964; De Ley and De Moor, 1977), vitamin A deficiency of the dam during pregnancy (Stegenga, 1964) and calf nutrition (whole milk versus milk powder) (Van Huffel et al., 1986) have all been investigated, but no statistically significant associations have been demonstrated. Straight hocks may be a predisposing factor for spastic paresis, but further research is necessary to confirm or to invalidate this statement (Coopman et al., 2000).

Ledoux (2004) suggested similarities between sub-acute transmissible spongiform encephalopathies and bovine spastic paresis. In both cases neuronal

vacuolation can be detected, although these lesions are not constant in or characteristic of spastic paresis. Nonetheless, Ledoux (2004) suggested that intra- and inter-species transmission studies should be undertaken to further investigate his hypothesis. Alternatively, Baird et al. (1974) hypothesized a viral origin for the disease because a mild non-suppurative encephalitis was detected in two calves, but no viruses were isolated. Furthermore, the absence of signs of an acute infectious disease makes an infectious aetiology of spastic paresis unlikely (Keith, 1981; Ledoux, 2001). However, so far no experimental inoculation trials have been performed to identify a putative prion, viral, bacterial or parasitic origin for spastic paresis.

An asymmetric hind limb paresis of cattle in New South Wales, which shares similarities with bovine spastic paresis, was suspected to be due to an "in-utero plant poisoning" but no specific plant association was established (Bourke, 1996).

Diagnostic protocol

Careful palpation and precise observation of the affected animal during rising, standing and at walk is the first step in the diagnosis of spastic paresis, but correct identification of all affected spastic muscles may not always be straightforward. Such identification is important, since the muscles affected greatly influence the outcome of surgical treatment (Touati et al., 2003). BSP-M cases, in particular, can resemble BSP-G cases. Animals affected with BSP-M that undergo selective tibial neurectomy can still have residual spastic contractions of quadriceps femoris or other muscle groups, and thus poor post-operative improvement.

Epidural injection of 0.38% procaine solution can resolve BSP-G symptoms after 10 to 15 minutes (De Ley and De Moor, 1980) but does not resolve symptoms associated with BSP-Q and BSP-M (Vertenten, 2006).

Compared with normal calves, electromyography studies on BSP-G calves have demonstrated significantly increased activity of the gastrocnemius and superficial digital flexor muscles in standing animals, but no other abnormalities. However, voluntary muscle activity can falsely contribute to the electromyographic read-out, hindering the objective identification of the involved muscles (Denniston et al., 1968; Bijleveld and Hartman, 1976).

There are no specific biochemical or haematological changes in spastic calves (Denniston et al., 1968; Bijleveld and Binkhorst, 1973; Baird et al., 1974; De Ley and De Moor, 1975). Plasma muscle enzyme levels are elevated, but this has been attributed to mild trauma resulting from the continuous muscle spasms when the animal is standing (Denniston et al., 1968; De Ley and De Moor, 1975).

Remodelling of the calcaneus is detectable using radiography with osteoporotic regions and exostoses visible at the dorsal aspect of the calcaneus. The epiphysis may be enlarged and irregular. Osteoporotic regions and exostoses may also be present at the level of the distal epiphysis of the tibia (Frederik and Van 't Hooft, 1962).

Bovine spastic paresis needs to be differentiated from a few other diseases and has similarities with the spastic syndrome that occurs more commonly in adult cattle and is characterised by episodic spasms of muscles in one or both hind limbs and sometimes in the back, rather than the progressive signs observed in cases of spastic paresis (Bradley and Wijeratne, 1980; Wells et al., 1987; Gentile and Testoni, 2006). Proximal patellar fixation, which is not often seen in young cattle, and congenital hyperextension of the tarsal joint, already present in newborn calves, differ in the absence of painful spastic muscle contractions and the age of onset (Baird et al., 1974; Vertenten, 2006).

Treatments

Due to the hereditary component of the disease, treatment of animals suffering from spastic paresis is of questionable value. In any case, treated animals should be banned from breeding. Several pharmaceutical and surgical treatments have been proposed. The limited evidence for medical treatments, such as lithium, means that surgical treatments are currently the only ones that are considered to be useful.

Transection of the superficial portion of the gastrocnemius tendon and half of the superficial flexor tendon was the first surgical treatment proposed (Götze, 1932). Hyperflexion of the tarsocrural joint often resulted in the calcaneus nearly touching the ground. Relief of muscle spasticity was often temporary as the cut tendon ends fused with scar tissue formation (Bouckaert and De Moor, 1966; Weaver, 1991). Pavaux et al. (1985) proposed a 'triple tenectomy', which included resection of both the superficial and deep branches of the gastrocnemius tendons and the tarsal tendon of the biceps femoris muscle and semitendinosus muscle, leaving the superficial flexor tendon intact. In some cases, the dense fascia caudal to the distal end of the tibia is transected (Pavaux et al., 1985). The advantage of this modified gastrocnemius tenectomy over the traditional tenectomy procedure is that 'dropped hock' does not occur after surgery (Weaver, 1991). However, there are no studies that have reported the outcome in large groups of animals.

Neurectomy of the tibial nerve or of its branches to the gastrocnemius has been developed as an alternative to tenotomy/tenectomy (Bouckaert and De Moor, 1966; Boyd and Weaver, 1967; Osinga and De Boer, 1973; De Ley and De Moor, 1977). In this technique, the tibial nerve is approached through a lateral incision between the two heads of the gluteobiceps muscle, its two branches to the gastrocnemius are identified and isolated, and a 3 cm section of each branch is resected. The caudal branch is constant in position and supplies the medial and axial parts of the gastrocnemius muscle. The oblique branch is more variable, but in most cases takes a lateral position in relation to the main part of the tibial nerve and innervates the lateral gastrocnemius head. The identification of the

two branches can be confirmed by mechanical or, less traumatically, electrical stimulation. Aberrant innervation of the gastrocnemius muscle may occur.

A retrospective study of selective tibial neurectomy in double-muscled Belgian Blue calves reported good results in 94/113 of the calves and considerable improvement in another five. In 14/113 animals, the long-term surgical results were poor (Vlaminck et al., 2000). Even though the function of the gastrocnemius muscle is lost, no ambulatory problems were observed after successful surgery since the superficial flexor tendon replaced the function of the denervated muscle. Rupture of the denervated muscle was reported in 5/113 cases. Bouisset et al. (1980) described an alternative approach with the tibial nerve being approached between the gluteobiceps and semimembranosus muscles. The branches innervating the gastrocnemius muscle were isolated just before they entered the muscle belly. As a consequence, electrical stimulation of the branches was not necessary. The large distance to the tibial nerve in double muscled calves is the main disadvantage of this approach.

Advantages of selective tibial neurectomy over tenectomy include fewer weight-bearing problems when precautions are taken to limit calf movement in the immediate postoperative period, and fewer recurrences. It is however technically more difficult to perform (Vlaminck et al., 2000). Complete tibial neurectomy has been described, but this results in decreased stability of the hind limb postoperatively (Bijleveld, 1973; Osinga and De Boer, 1973; Weaver, 1997).

So far, no adequate treatment of BSP-Q and BSP-M has been described.

Neurectomy or chemical destruction of the femoral nerve is not feasible as it may induce a total paralysis of the quadriceps femoris muscle, making the animal unable to bear weight on the limb. In human beings, a selective rhizotomy, which destroys the dorsal spinal nerve roots which communicate with the muscle, can be used to reduce spasticity without inducing a paralysis of the affected muscle (Roberts, 2013). This technique has already been used experimentally in three animals suffering from BSP-G in an attempt to elucidate the pathology of the disease (De Ley and De Moor, 1977); however, since tenectomy and neurectomy are easier and quicker to perform, this technique has not been used to treat BSP-G.

References

- Arnault G., 1979. Etude de la parésie spastique des bovins. Rivation et Cie, Bobigny, France.
- Arnault G., 1982. Bovine spastic paresis. An epidemiological, clinical and therapeutic study in a Charolese practice in France. Efficacy of Lithium Therapy. Proceedings of the XIIth World Congress of Diseases of Cattle, Amsterdam, The Netherlands, 1982, 853-858.
- Baird J.D., Johnston K.G., Hartley W.J., 1974. Spastic paresis in friesian calves. Australian Veterinary Journal 50, 239-245.
- Baker J., Ciszewski D., Lowrie C., Mullaney T., 1989. Spastic paresis in pygmy goats. Journal of Veterinary Internal Medicine 3, 113.
- Barone R., 2000. Anatomie Comparée des Mammifères Domestiques - Tome 2, Arthrologie & Myologie. 4ed. Vigot, Paris, France, p 1021.
- Bijleveld K., 1973. De behandeling van spastische parese bij het rund door middel van een totale neurectomie van de N.tibialis. Tijdschrift voor Diergeneeskunde 98, 791-794.
- Bijleveld K., Binkhorst G.J., 1973. Onderzoek van bloed en liquor cerebrospinalis bij kalveren met spastische parese. Tijdschrift voor Diergeneeskunde 98, 803-806.
- Bijleveld K., Hartman W., 1976. Electromyographic studies in calves with spastic paresis. Tijdschrift voor Diergeneeskunde 101, 805-808.
- Bouckaert J.H., De Moor A., 1966. Treatment of spastic paralysis in cattle: improved denervation technique of the gastrocnemius muscle and post-operative course. Veterinary Record 79, 226-229
- Bouisset S., Pavaux C., Daviaud L., 1980. Jarret droit: autre voie d'accès pour une névrectomie. Le Point Vétérinaire 10, 43-48.

- Bourke C.A., 1996. Bovine asymmetric hind limb paresis, a presumptive in-utero plant poisoning. *Australian Veterinary Journal* 74, 42-45.
- Boyd J.S., Weaver A.D., 1967. Spastic paresis in cattle. *Veterinary Record* 80, 529-530.
- Bradley R., Wijeratne W.V.S., 1980. A locomotor disorder clinically similar to spastic paresis in an adult Friesian bull. *Veterinary Pathology* 17, 305-315.
- Browning G.F., Rawlinson R.J., Begg A.P., 1986. Spastic paresis in a Poll Hereford heifer. *Australian Veterinary Journal* 63, 367-369.
- Budras K., Habel R.E., 2003. *Bovine Anatomy. An illustrated Text.* Schlütersche, Hannover, pp 17.
- Burgoyne R.D., 2007. Neuronal calcium sensor proteins: generating diversity in neuronal Ca²⁺ signaling. *Nature Reviews Neuroscience* 8, 182-193.
- Chiocchetti R., Grandis A., Bombardi C., Joechler M., Pisoni L., Gentile A., 2006. Bulbo-Pons-Mesencephalic Projections to the Lumbo-Sacral Spinal Cord in Healthy and Spastic Paresis-Affected Cattle: A Comparative Investigation. *Veterinary Research Communications* 30, 175-177.
- Chomiak M., Szteyn S., 1970. Veränderungen im zentralen Nervensystem bei einem Fall von spastischer Parese des Rindes. *Schweizer Archiv für Tierheilkunde* 112, 397-401.
- Chomiak M., Lakomy M., Szteyn S., Milart Z., 1972. Weitere histopathologische Untersuchungen des Zentralnervensystems bei der Paresis spastica des Rindes. *Archiv für experimentelle Veterinärmedizin* 27, 791-798.
- Coopman F., Peelman L., Van Zeveren A., 2000. Overerving van spastische parese, normale en afwijkende spronghoeken en hun onderlinge verband in het belgisch witblauw ras: een kritische benadering. *Vlaams Diergeneeskundig Tijdschrift* 69, 249-257.
- Dawson P.L., 1975. The economic aspect of spastic paresis of the hind limbs of Friesian cattle. *Veterinary Record* 97, 432-433.

Introduction

- De Ley G., De Moor A., 1975. Bovine Spastic Paralysis: Cerebrospinal Fluid Concentrations of Homovanillic Acid and 5-Hydroxyindoleacetic Acid in Normal and Spastic Calves. *American Journal of Veterinary Research* 36, 227-228.
- De Ley G., De Moor A., 1977. Bovine spastic paresis: results of surgical desafferentation of the gastrocnemius muscle by means of spinal dorsal root resection. *American Journal of Veterinary Research* 38, 1899-1900.
- De Ley G., De Moor A., 1980. Bovine spastic paralysis: results of selective γ -efferent suppression with dilute procaine. *Veterinary Science Communications* 3, 289-298.
- De Moor A., Bouckaert J.H., Top W., 1964. Chirurgische behandeling van spastische parese bij het rund door denervatie van de m. gastrocnemius. *Vlaams Diergeneeskundig Tijdschrift* 33, 1-18.
- Denniston J.C., Shive R.J., Friedli U., Boucher W.B., 1968. Spastic paresis syndrome in calves. *Journal of the American Veterinary Medical Association* 152, 1138-1149.
- Dewulf M., De Moor A., Cockelbergh D., 1982. Spastische Parese bij het rund: Vergelijkende studie van het gehalte aan dopamine en noradrenaline in verschillende hersengebieden. *Vlaams Diergeneeskundig Tijdschrift* 51, 433-437.
- Fasquelle C., Sartelet A., Wanbo L., Dive M., Tamma N., Michaux C., Druet T., Huijbers I.J., Isacke C.M., Coppieters W., Georges M, Charlier C, 2009. Balancing selection of a frame-shift mutation in the MRC2 gene accounts for the outbreak of the Crooked Tail Syndrome in Belgian Blue Cattle. *PLoS Genetics* 5, e1000666.
- Formston C., Jones E.W., 1956. A spastic form of lameness in Friesian cattle. *Veterinary Record* 68, 624-627.
- Frederik G.H., Van 't Hooft A.J.G., 1962. Spastische parese bij het M.R.IJ. veeslag en enige röntgenologische aspecten daarvan. *Tijdschrift voor Diergeneeskunde* 87, 699-708.
- Gadgil B.A., Agarwal S.P., Patel U.G., 1970. Spastic paresis in adult Indian cattle. *Veterinary Record* 86, 694-697.

- Gentile A., Pittoni S., Famigli Bergamini P., 2002. La Paresi Spastica nel bovino di razza Romagnola. *Atti della Societa Italiana di Buiatria* 34, 191-198.
- Gentile A., Testoni S., 2006. Inherited disorders of cattle: a selected review. *Slovenian Veterinary Research* 43, 17-29.
- Götze R., 1932. Spastische Paresis der Hinteren Extremität bei Kälber und Jungrindern. *Deutsche Tierärztliche Wochenschrift* 40, 197-200.
- Griffiths I.B., 2005. Spastic paresis in calves. *The Veterinary Record* 157, 786-787.
- Harper P.A., 1993. Spastic paresis in Brahman crossbred cattle. *Australian Veterinary Journal* 70, 456-457.
- Hanset R., Serteyn D., Grobet L., Michaux C., 1993. The bovine spastic paresis and its inheritance. *Annales de Médecine Vétérinaire* 137, 237-247.
- Keith J.R., 1981. Spastic paresis in beef and dairy cattle. *Veterinary Medicine & Small Animal Clinician* 76, 1043-1047.
- Ledoux J.-M., 2001. Bovine spastic paresis: etiological hypotheses. *Medical Hypotheses* 57, 573-579.
- Ledoux J.-M., 2004. Hypothesis of interference to superinfection between bovine spastic paresis and bovine spongiform encephalopathy; suggestions for experimentation, theoretical and practical interest. *Medical Hypotheses* 62, 346-353.
- Legendre P., 2001. The glycinergic inhibitory synapse. *Cellular and Molecular Life Sciences* 58, 760-793.
- Leipold H.W., Huston K., Guffy M.M., Noordsy J.L., 1967. Spastic paresis in beef shorthorn cattle. *Journal of the American Veterinary Medical Association* 151, 598-601.
- Lewandowski M., Szteyn S., Chomiak M., 1970. Ein Fall von spastischer Lähmung (Paresis spastica) beim Stier. *Wiener tierärztliche Monatsschrift* 57, 106-109.
- Love J., Weaver A.D., 1963. Spastic paresis in a crossbred shorthorn steer. *Veterinary Record* 75, 394-396.

Introduction

- Mayhew I.G., 2009. Large Animal Neurology. 2nd ed., Wiley-Blackwell, Oxford, United Kingdom, pp 453.
- Milart Z., Chomiak M., 1971. Pathological Changes in the Central Nervous System of a Bull Affected with Spastic Paresis. *The Veterinary Record* 88, 66-68.
- Miura M., Fukuda N., Ueki A., Ikegaya A., Ikeda A., Anan T., Takehana K., Yamaguchi E., Kim D., Sato S., Yamagishi N., 2009. Spastic paresis in a Japanese Black calf. *Japanese Journal of Veterinary Clinics* 32, 8-11.
- Osinga A., De Boer G., 1973. Field experience of partial and total neurectomy of the tibial nerve in spastic paresis of calves. *Tijdschrift voor Diergeneeskunde* 98, 795-798.
- Pariset L., Bongiorno S., Bueno S., Gruber C.E.M, Prosperini G., Chillemi G., Bicorgna S., Gentile A., Valentini A., 2013. Microarray gene expression profiling of neural tissues in bovine spastic paresis. *BMC Veterinary Research* 9, doi:10.1186/1746-6148-9-122.
- Pavaux C., Sautet J., Lignereux J.Y., 1985. Anatomy of the bovine gastrocnemius muscle as application to the surgical correction of spastic paresis. *Vlaams Diergeneeskundig Tijdschrift* 54, 296-312.
- Rasbech N.O., 1963. Spastic paresis of the hind limbs of the offspring from bulls of the Red Danish milk breed. In: *Proceedings of 9th Nordic Veterinary Congress, Copenhagen, 1963*, 519.
- Roberts S.J., 1965. Hereditary spastic diseases affecting cattle in New York State. *Cornell Veterinarian* 55, 637-644.
- Roberts A., 2013. Surgical management of spasticity. *Journal of Children's Orthopaedics* 7, 389-394.
- Stegenga T., 1964. Aangeboren afwijkingen bij rundvee. *Tijdschrift voor Diergeneeskunde* 89, 286-293.
- Szteyn S., Chomiak M., Lewandowski M., 1972. A contribution to Paresis Spastica in Cattle. *Zentralblatt für Veterinärmedizin Reihe A* 19, 477-481.

- Thomason K.J., Beeman K.B., 1987. Spastic Paresis in Gelbvieh calves: an examination of two calves. *Veterinary Medicine* 82, 548-553.
- Touati K., Muller P., Grulke S., Peters F., Sertheyn D., 2003. La parésie spastique du quadriceps fémoral: Une nouvelle entité clinique chez le veau de race Blanc Bleu Belge. *Annales de Médecine Vétérinaire* 147, 261-265.
- van Gastel-Jansen A., Frederik G.H., 1962. Pathological changes in the tarsi of cattle suffering from paresis spastica, examined by means of X-Rays. *Veterinary Record* 74, 1260-1263.
- Van Huffel X., Demoor A., Lampo P., 1986. Bovine spastic paresis - Genetic and environmental factors. *Vlaams Diergeneeskundig Tijdschrift* 55, 21-30.
- Vertenten G., 2006. Recente evolutie van spastische parese bij het kalf. *Het Dierenartsen Weekblad* 44, 14-17.
- Vlaminck L., De Moor A., Martens A., Steenhaut M., Gasthuys F., Desmet P., Van Branteghem L., 2000. Partial tibial neurectomy in 113 Belgian blue calves with spastic paresis. *Veterinary Record* 147, 16-19.
- Weaver A.D., 1991. Modified gastrocnemius tenectomy: a procedure to relieve spastic paresis in dairy calves. *Veterinary Medicine & Small Animal Clinician* 86, 1234-1239.
- Weaver A.D., 1997. Spastic paresis; In: Greenough, P.R., Saunders, W.B. (ed): *Lameness in cattle*, 3ed., W.B. Saunders Co, Philadelphia, Pennsylvania, USA, pp 213-215.
- Wells G.A., Hawkins S.A., O'Toole D.T., Done S.H., Duffell S.J., Bradley R., Hebert C.N., 1987. Spastic syndrome in a Holstein bull: a histologic study. *Veterinary Pathology* 24, 345-353.
- Wheat J.D., 1960. Spastic lameness of the hindlimbs of an Aberdeen Angus heifer. *Journal of the American Veterinary Medical Association* 137, 659-660.

CHAPTER 2

Scientific aims

Over the last decade, a higher prevalence of atypical entities of spastic paresis was observed in the large animal clinic of Ghent University. Based on clinical symptoms, differentiation between BSP entities is not always straightforward. Moreover, animals with an atypical condition that undergo selective tibial neurectomy will often only partially improve and in some cases, symptoms will even worsen after surgery.

The **overall aim** of this thesis was to optimise the diagnosis and treatment of calves affected with an atypical entity of spastic paresis involving the quadriceps femoris muscle (BSP-Q and BSP-M). Therefore, 3 main research objectives were formulated:

The **first objective** was to study the long-term outcome of calves suffering from atypical presentations of spastic paresis after surgical intervention and/or conservative management. The results of this study should contribute to the selection of those cases of spastic paresis that are suitable candidates for selective tibial neurectomy and those that are not.

The **second objective** was to develop a regional anaesthesia technique to objectively evaluate quadriceps femoris muscle involvement in spastic calves. Different approaches to perform regional anaesthesia of the femoral nerve were developed and their accuracy was tested in a cadaver study. The best technique was evaluated in a clinical trial including both normal and spastic calves.

The **third objective** was to assess selective dorsal rhizotomy as a surgical treatment of quadriceps femoris muscle spasticity. In order to verify the effectiveness of the rhizotomy procedure, a technique for recording of cord dorsum potentials after stimulation of the saphenous nerve needed to be standardized in calves.

CHAPTER 3

Long-term outcome of conservative management or surgical treatment of bovine spastic paresis: 79 cases

Adapted from:

De Vlaminck C., Pille F., Vlaminck L., 2015. Long-term outcome of conservative management or surgical treatment of bovine spastic paresis: 79 cases.

Veterinary Surgery, accepted.

Summary

The long-term clinical outcome in treated and non-treated calves suffering from bovine spastic paresis of the gastrocnemius (BSP-G) or the quadriceps femoris muscle (BSP-Q) and with mixed muscle involvement (BSP-M) is reported by means of a retrospective study.

Medical records of 79 calves treated by selective tibial neurectomy or conservatively managed for bovine spastic paresis were analyzed for sex, breed, lineage history and the onset, duration and severity of clinical signs. Cases were classified as unilateral or bilateral BSP-G, BSP-Q or BSP-M. Long-term follow-up information was obtained by telephone questionnaire.

The study group included 26 BSP-G (33%), 16 BSP-Q (20%) and 37 BSP-M (47%) cases. BSP-M calves and BSP-Q calves were significantly more bilaterally affected, in contrast to BSP-G calves ($p=0.04$, $p< 0.001$ respectively). Twenty-five of the 26 BSP-G animals underwent surgery, which resulted in 86% of the cases in a complete resolution of clinical signs. Twenty-nine of the 37 BSP-M animals underwent surgery; none of them recovered completely but the majority improved (81.5%). Clinical signs gradually worsened in all the non-treated BSP-M animals. None of the BSP-Q calves were treated. In 2/3 of the BSP-Q calves the clinical signs gradually worsened whereas 1/3 of the animals improved spontaneously.

Conclusions: Selective tibial neurectomy is advocated for the treatment of BSP-G and in selected cases of BSP-M. However, in the latter group this will only result in partial resolution of clinical signs. No surgical treatment exists for BSP-Q calves although spontaneous improvement is possible.

Introduction

Bovine spastic paresis is a well-known progressive neuromuscular disease in cattle. Even though the aetiology and pathology are not yet completely understood, it is assumed that an overactive stretch reflex causes overstimulation of the gamma motor neurons, resulting in repetitive muscle spasms in one or both hind limbs (De Ley and De Moor, 1980). Original reports describe bovine spastic paresis of the gastrocnemius muscle (BSP-G) in which the affected hind limb is typically stretched in a caudal direction because of gastrocnemius muscle spasms (Hamoir, 1922; Baird et al., 1974; Keith, 1981). Clinical signs appear as soon as the animal is standing. Economic losses are due to decreased weight gain and early slaughter of affected animals. Surgical treatment options such as triple tenectomy or (selective) tibial neurectomy have been described with success rates of more than 80% (Bouckaert and De Moor, 1966; Weaver, 1991; Vlaminck et al., 2000; Barvalia and Patil, 2003).

More recently, involvement of other muscle groups was reported (Touati et al., 2003; Vertenten, 2006). Spastic contractions of the quadriceps femoris muscle group (BSP-Q) result in a cranially directed pendulous movement of the affected hind limb. Later, involvement of the gastrocnemius and/or the quadriceps femoris muscle and/or potentially other muscle groups of the hind limb was observed (BSP-M) causing cranially, caudally or laterally directed movements of the affected hind limb depending on the dominant spastic muscle group. For these animals, no specific therapy exists nor have there been reports published to document the evolution of their clinical signs and its consequences.

This paper presents a long-term retrospective analysis of conservative management or surgical treatment by selective tibial neurectomy in 79 calves affected by different entities of the spastic paresis syndrome. It was hypothesized that the outcome for selective tibial neurectomy in BSP-M affected calves would be less favorable than the outcome in surgically treated BSP-G calves.

Materials and methods

Cases

Medical records of calves affected by bovine spastic paresis, presented at the veterinary clinic of Ghent University between September 2009 and December 2013, were reviewed. Classification as BSP-G, BSP-M or BSP-Q was primarily based on clinical examination including close observation of each animal while changing from sternal decubitus to a standing position followed by ambulation on a solid surface. The main parameters to classify the calves as BSP-G, BSP-M or BSP-Q were the direction in which the hind limb(s) was/were stretched and the muscle tone intensity during palpation. Calves were classified as BSP-G calves when the hind limb(s) was (were) stretched in a caudal direction and when an increased muscle tone of the gastrocnemius muscle was palpated while standing. BSP-M calves stretched their hind limb(s) in a varying direction (cranial, caudal, lateral) and an increased muscle tone of several muscles of the hindquarters was palpated. Calves were classified as BSP-Q calves when the non-weight bearing hind limb was stretched cranially while standing with a convexely arched back. Upon palpation, increased quadriceps femoris muscle tone was palpated. When walking, the hind limbs were rigidly advanced with a swinging pendulum motion, resembling the gait of a tin soldier. The degree of spasticity was graded as mild, moderate or severe (Table 1) and uni- or bilateral involvement was recorded. Other data that were collected included sex, age at onset of clinical signs, age at admission and breed. Owners were asked whether they had noticed comparable clinical signs in the parents of the patient, or in their parents' offspring.

Table 1 - Severity of spastic clinical signs at the moment of admission.

Severity	Description clinical signs
Mild	Repetitive spastic motion only visible shortly after rising, then changing to a hyperextended position of the tarsus and/or later to continuous weightshifting Calf able to stand > 5 minutes
Moderate	Repetitive spastic motion continuously visible while standing and ambulating Calf able to stand > 5 minutes
Severe	Repetitive spastic motion continuously visible while standing and ambulating Calf able to stand < 5 minutes

Treatment

BSP-Q animals were given a poor prognosis because no surgical treatment was available. These animals were euthanized or returned to the farm for conservative management which included individual stall confinement and providing easy access to food and water. BSP-G calves and BSP-M calves in which the spasticity was predominantly caudally directed, indicating obvious and significant gastrocnemius involvement, were amenable for surgery.

Surgical treatment consisted of a selective tibial neurectomy as described by Bouckaert and De Moor (1966). In case of bilateral hind limb involvement the limb that first showed spastic contractions at the moment of getting up was consistently determined as being the most severely affected and was chosen for surgery. None of the animals was treated bilaterally in one surgical procedure to reduce the risk of postoperative gastrocnemius muscle rupture.

Calves were sedated with xylazine hydrochloride (0.1 to 0.2 mg/kg intramuscularly (IM); Xyl-M 2%, VMD, Belgium). Caudal epidural anaesthesia with procaine 4% was administered to recumbent calves to obtain complete paralysis of the hind limbs. The calves were positioned in lateral recumbency with the affected limb uppermost. After surgical preparation, a 15 cm skin incision was made between the two parts of the gluteobiceps muscle. These two latter parts were bluntly separated. The fascia genus was incised to expose the underlying blood vessels and nerves. The tibial nerve was identified by its anatomical location as it passes between the medial and lateral part of the gastrocnemius muscle. After nerve isolation and dissection of its epineurium, individual branches were electrically stimulated. The two branches corresponding with the gastrocnemius muscle bellies were identified based on muscle contraction following stimulation. Three centimeters of each nerve branch were resected. The two parts of the gluteobiceps muscle were sutured with absorbable suture material (Surgicryl USP 2, SMI, Belgium) in a continuous pattern. The skin was closed with a continuous Ford interlocking pattern (Surgicryl USP 2). Penicillin (Peni-Kel 300.000 IE/ml, Kela, Belgium, 21.000 IE/kg intramuscularly) and meloxicam (Metacam, Boehringer-Ingelheim, Belgium, 0.5 mg/kg subcutaneously) were administered systemically during surgery. No medical treatment was continued after surgery. All patients stayed in the clinic for maximum 2 days post-operatively. After discharge from the clinic farmers were advised to keep their calf confined to a small box with soft surface for 1 month to prevent inadvertent gastrocnemius muscle rupture. After this period, bilaterally affected cases were amenable for surgery in their contralateral hind limb.

Follow-up

Follow-up was performed through a telephone questionnaire after a minimum period of three months. Farmers of surgically treated calves were asked to evaluate the result of surgery and based on this information a good, moderate or bad outcome was assigned. A good outcome was assigned when no spastic contractions in the treated limb were visible anymore. A moderate outcome was defined as a clear improvement of clinical signs in the treated limb even though some residual spasticity was present. A bad outcome was given to animals

without improvement of clinical signs in the treated limb or when spasticity in this limb progressed, causing these animals to remain recumbent for longer periods as compared to before surgery. In cases with bilateral involvement in which only one of both hind limbs was surgically treated, the farmer was also asked to describe the evolution of spasticity in the untreated limb. Finally, the farmer was also asked to evaluate weight gain in comparison to healthy animals of the same age. In conservatively managed cases, the same scores (good, moderate, bad) as for surgically managed cases were used and their weight gain was evaluated.

Statistics

Statistical analyses were performed with statistical software SPSS Statistics 22 (IBM, Brussels, Belgium). Pearson's chi square test was used to evaluate the prevalence of the different presentations of the disease between sexes and to evaluate the prevalence of unilateral and bilateral affected animals. Single-factor analysis of variance was performed to evaluate the symptom severity and the age of onset between entities. A value of $P < 0.05$ was considered significant for all analyses.

Results

During the period September 2009 – December 2013, 79 calves affected with bovine spastic paresis were admitted. The majority of the calves (77/79) were Belgian Blue, one was a Holstein Friesian and one was a zebu. Based on their clinical signs 26 animals were classified as BSP-G (33%), 16 as BSP-Q (20%) and 37 as BSP-M (47%) cases. The Holstein Friesian and the zebu both suffered from BSP-G. In each year of the study, the majority of referred calves with spastic paresis showed an atypical presentation of the disease (BSP-Q and BSP-M together). Only a minority of the referred calves demonstrated a solitary spasticity of the gastrocnemius muscle (Figure 4).

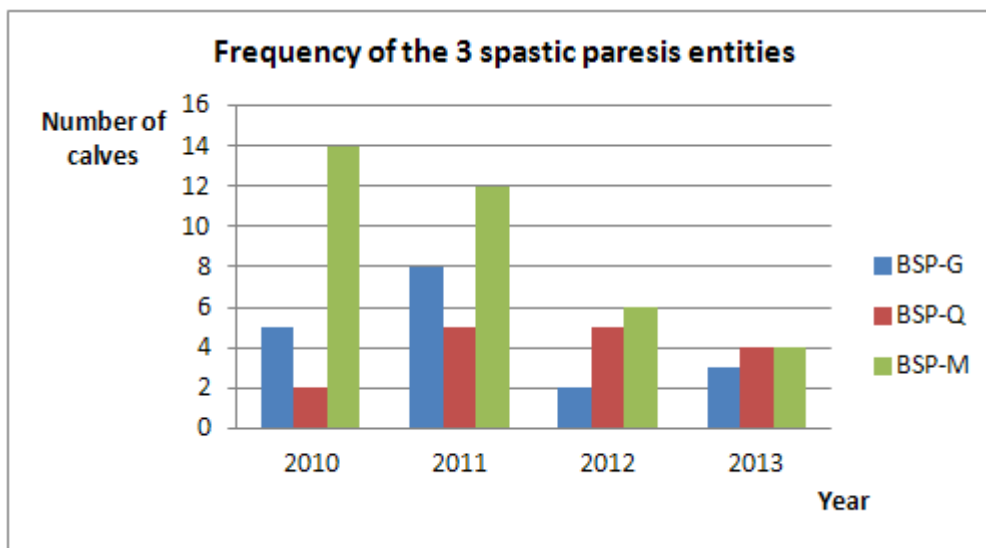


Figure 4 - Frequency of the 3 spastic paresis entities during the study. In each year, the majority of referred calves is suffering from an atypical presentation of BSP (BSP-Q and BSP-M together). A minority of the referred calves showed the typical BSP-G presentation in the last two years of the study.

No sex predilection was observed for any of the three different entities of spastic paresis ($p = 0.45$, $p = 1$, $p = 0.22$ respectively). BSP-M calves and BSP-Q calves were significantly more bilaterally affected ($p = 0.04$, $p < 0.001$ respectively), in contrast to BSP-G calves ($p = 0.20$). The level of spasticity was scored mild in 11

(14%), moderate in 20 (25%) and severe in 48 (61%) cases. No significant differences in symptom score were observed between entities ($p=0.49$).

Parent animals were affected by spastic paresis in 10 cases (6 bulls, 4 cows). In 12 cases, other offspring of parent animals also demonstrated spastic paresis symptoms.

The mean age at which the owner noticed the clinical signs the first time was 90 days (range 1 - 670 days) in BSP-G calves, 14 days (range 1 - 180 days) in BSP-Q calves and 44 days (range 1 - 300 days) in BSP-M calves. The mean age of first clinical signs was not significantly different between entities ($p= 0.17$). The median age at admission of calves suffering from BSP-G was 128 days (range 20 - 733 days), 50 days (range 9 - 590 days) for BSP-Q calves, and 143 days (range 13 - 289 days) for BSP-M calves.

Outcome following surgical treatment

Overall, selective tibial neurectomy was performed in 54 animals (68 %) including 25 BSP-G and 29 BSP-M cases. Five cases were lost for follow-up. After a median follow-up period of 179 days (range 90 - 720), the outcome was evaluated to be good for 19 calves (38.8%), moderate for 23 calves (47 %) and bad for 6 calves (12.2 %).

OUTCOME AFTER SURGERY IN BSP-G CALVES

All BSP-G animals were operated except the zebu for which the owner declined any further financial expenses. Long-term follow-up (median 185 days; range 90 - 570) was available for 22 of 25 surgically treated animals (Table 2). Nineteen of them (86.4 %) had a good outcome, two had a moderate outcome, and one had a bad outcome. Weight gain was considered comparable to the farm's peer group in twenty animals (90.1%) including the 19 animals cured from spasticity following surgery and one animal with persistent spasticity at a lower level. One unilaterally affected calf with a moderate outcome and 1 unilaterally affected calf with a bad outcome showed obvious growth recession. Postoperative complications were encountered in one calf which developed a dropped hock shortly after surgery, probably due to a rupture of the gastrocnemius muscle and

the superficial digital flexor tendon. Cast immobilization of the hock for 1 month provided good healing and finally resulted in a good outcome and normal weight gain.

Table 2 - Outcome after surgery in 22 treated BSP-G calves after a median period of 185 days and in 27 treated BSP-M calves after a median period of 169 days.

Spasticity degree at the moment of admission	Outcome after surgery					
	Good		Moderate		Bad	
	BSP-G	BSP-M	BSP-G	BSP-M	BSP-G	BSP-M
Mild	5	-	-	2	-	-
Moderate	5	-	-	8	-	2
Severe	9	-	2	12	1	3
Total	19	-	2	22	1	5

Ten out of 22 calves were bilaterally affected. Only one was operated on both limbs with a 4 weeks interval in between. Unfortunately, the calf died two weeks after the second surgery for reasons unrelated to surgery or spasticity. In 3 of the other 9 bilaterally affected animals, spastic contractions in the non-treated limb were less obvious 1 month after surgery. In 5 animals the spasticity level did not change and in 1 animal the spasticity progressively worsened over time.

One surgically treated animal that was unilaterally affected at the time of admission developed spasticity in the non-affected limb two months postoperatively and was not further treated. Spasticity worsened over time to a severe level necessitating early slaughter in this animal.

OUTCOME AFTER SURGERY IN BSP-M CALVES

Twenty-nine of the 37 BSP-M animals (78 %) underwent surgery. Long-term follow-up was available for 27 animals after a median period of 169 days (range 90 – 720) (Table 2). None of these calves had a good outcome after surgery. In 22/27 calves (81.5%) spasticity persisted but at a lower level (moderate outcome). All 22 animals were considered to grow slower in comparison with a

farm's peer group. In 5/27 animals (18.5%), a deterioration of the clinical signs was observed; hence the outcome was evaluated as bad. For all of these 5 animals, severe weight recession was observed.

Two calves developed a dropped hock shortly after surgery, probably due to a rupture of the gastrocnemius muscle and the superficial digital flexor tendon. This healed after cast immobilization of the hock in one calf. The other animal was euthanized because the farmer declined further treatment.

Eighteen of 27 calves (67 %) were bilaterally affected. None of the 18 calves were operated on both limbs. Farmers were unable to evaluate the evolution of spasticity in the untreated limb.

Outcome following conservative management of BSP-M and BSP-Q cases

Eight calves classified as BSP-M were managed conservatively because the gastrocnemius muscle seemed not predominantly involved in the disease process. None of these calves were euthanized immediately after diagnosis, even though a poor prognosis was given. Follow-up information was available in all animals after a median period of 196 days (range 90 – 635) (Table 3). In all animals clinical signs progressed to a severe level necessitating early slaughter.

Table 3 - Outcome after conservative management in 8 BSP-M calves after a median period of 196 days and in 12 BSP-Q calves after a median period of 121 days.

Spasticity degree at the moment of admission	Outcome after surgery					
	Good		Moderate		Bad	
	BSP-M	BSP-Q	BSP-M	BSP-Q	BSP-M	BSP-Q
Mild	-	-	-	1	1	-
Moderate	-	-	-	3	-	2
Severe	-	-	-	-	7	6
Total	-	-	-	4	8	8

Three of 16 calves diagnosed with bilateral BSP-Q were euthanized in the clinic. One of them had mild clinical signs and 2 showed a severe spasticity. Long-term follow-up information was available for the remaining 13 animals following a period of 121 days (range 90 – 280) (Table 3). One calf died from a reason unrelated to the spastic paresis. The other 12 animals were all bilaterally affected. Eight of these 12 animals (66.7%) were slaughtered within a short term after diagnosis because of symptom progression and failure to gain weight. In the other 4 out of these 12 animals (33.3%), the clinical signs gradually improved resulting in satisfying weight gain. At the time of telephone inquiry, 2 of the 4 calves were sold for slaughter at their desired weight. One animal was still at the farm with some residual spasticity two years after its diagnosis, and the fourth animal was pregnant.

Discussion

In this study 25 out of 26 BSP-G animals underwent surgery, which resulted in 86% of the cases in a complete resolution of clinical signs. Twenty-nine of the 37 BSP-M animals underwent surgery; none of them completely recovered but the majority improved (81.5%). In all the non-treated BSP-M animals, the clinical signs gradually worsened. None of the BSP-Q calves were treated. In 2/3 of the BSP-Q calves the clinical signs gradually worsened whereas 1/3 of the animals spontaneously improved.

The aetiology of spastic paresis is unknown, and it is generally believed that the disease is heritable but the genetic mechanisms may be complex. Up to now, it has not been possible to definitively determine the mode of inheritance (dominant or recessive) or the entity of penetrance of the single or multiple genes responsible. Strong evidence of a recessive mode of inheritance with low or incomplete penetrance was found (Leipold et al., 1967; Gadgil et al., 1970). Also in this study, in some cases spasticity was noticed in one of the parents or in the parents' offspring. One animal was part of a twin; however, its litter mate did not develop any spasticity.

Because of the heritability of the disease, affected animals should be banned for breeding purposes. Only when the owners keep the calves for beef, treatment is economically and ethically justified. However, with atypical presentations of the disease, it was shown in the present study that almost all animals were considered to grow slower in comparison with a farm's peer group. Nevertheless, some of the farmers admitted they did/will not ban their calf for breeding purposes, mainly because of the superior muscularity. With this attitude towards the disease, bovine spastic paresis will possibly never be eradicated.

Surgical treatment consisting in eliminating the spastic gastrocnemius contractions is unsatisfying in BSP-M and BSP-Q cases. As these are the majority of the animals admitted with spastic paresis nowadays at the authors' facilities, further research is needed to develop new treatment options. However,

establishing selection protocols may help to decrease the prevalence of this neuromuscular disorder.

As a treatment of BSP-G, a tenectomy of both the superficial and deep portion of the gastrocnemius tendon, and the tarsal tendon of the biceps femoris and semitendinosus muscles or a selective neurectomy of the branches of the tibial nerve supplying the gastrocnemius muscles is commonly performed. For both treatments a good outcome is reported. In our study, the overall percentage of calves with no residual spasticity after surgery in the treated limb was 38.8% which is notably lower than the outcome of 83.2 % after selective tibial neurectomy in the study of Vlaminck and others (2000). This less successful outcome can be explained by the fact that in 47% of our surgically treated patients other muscle groups than the gastrocnemius muscle were involved in the disease process. After denervation of the gastrocnemius muscle spasticity persists in the other affected muscle groups. The study of Vlaminck and others (2000) only included BSP-G calves. When only focusing on the BSP-G group in the present study, 86.4% of the animals had a good outcome following surgery which corresponds better to the study of Vlaminck and others (2000). Hence, identification of the muscle groups involved in this disease and differentiation between entities is imperative to formulate a success rate for selective tibial neurectomy.

Three calves developed a dropped hock shortly after surgery, probably due to a rupture of the gastrocnemius muscle and the superficial digital flexor tendon. Literature about this complication after surgery is lacking. The authors hypothesize that excessive physical force shortly after surgery and a defective proprioception of the denervated muscle may be the cause for a gastrocnemius rupture. Moreover, it is known that the superficial digital flexor tendon lies between the heads of the gastrocnemius muscle and is fused with the lateral head at its origin from the supracondylar fossa (Budras and Habel, 2003). So, the muscle body of the superficial digital flexor tendon may also rupture as the muscle heads of the gastrocnemius rupture, causing a dropped hock.

Careful palpation and precise observation of the affected animal during rising, standing and at walk is the first step in the diagnosis of spastic paresis. However, correct identification of all affected spastic muscles may not always be as

straightforward but greatly influences outcome associated with known surgical treatments aimed at gastrocnemius involvement (Touati et al., 2003).

In literature there is no consensus about the prevalence of spastic paresis in the two sexes. Griffiths (2005) reported a significantly higher prevalence of spastic paresis in female animals, while Ledoux (2001) states that the disease is more frequently seen in male calves. In our population no significant difference in gender distribution in any of the presentations of spastic paresis was observed.

BSP-Q is only reported in Belgian Blue calves and in the Italian double-muscled Romagnola breed (Gentile and Testoni, 2006). Up to now, BSP-M has only been reported in the Belgian Blue breed. To the authors' knowledge reports describing the BSP-Q and BSP-M presentations in other bovine breeds have not been published yet.

A significant difference in age of onset between calves suffering from BSP-Q and BSP-G has been described (4 weeks resp. 4 months) (Touati et al., 2003). However, in the present study, this difference in age of onset between the three entities was not significant. In all presentations a remarkable spreading was observed in the age at which the first clinical signs were noticed. This is probably due to the difference in age of onset between animals, but also to the difference in watchfulness between owners. It can be concluded that this parameter is rather unreliable to make a distinction between entities.

Only 1 of the 10 bilaterally affected BSP-G calves and none of the 18 bilaterally affected BSP-M calves returned after a month for a second operation, probably for financial reasons or because of a reduction of clinical signs in the untreated limb.

Even though bovine spastic paresis is known as a disease in which the clinical signs progressively aggravate, a reduction of spasticity in the untreated limb was reported in 26% of bilaterally affected BSP-G animals that underwent unilateral surgery (Vlaminck et al., 2000). In our study a comparable observation was made as 3/9 (33%) bilaterally affected BSP-G animals had an amelioration of clinical signs in the untreated limb. In bilaterally affected BSP-M animals, this reduction was not observed by the farmer. Farmers' inexperience in interpreting clinical signs might have influenced these findings. As the pathogenesis of the

disease is still not totally clear, giving an explanation for these observations remains difficult.

In 25% of the conservatively treated BSP-Q animals, the farmer reported amelioration over time in both limbs, even though a residual spasticity was still present. Two years after admission two of these calves were still alive, they grew well and the discomfort of the spastic contractions seemed acceptable. Hence, the prognosis of this entity may not be depicted so badly as was thought before, as in some cases an amelioration of the clinical signs is possible without further treatment.

To date, adequate surgical treatment of BSP-Q and BSP-M cases is not available. In some BSP-M calves, selective tibial neurectomy reduces spasticity and improves ambulatory possibilities although discomfort due to continued spasms of other muscle groups persists. As the quadriceps femoris muscle fixates the knee, this muscle is indispensable to be able to bear weight. Neurectomy or a chemical destruction of the femoral nerve may induce a total paralysis of the quadriceps femoris muscle, making the animal unable to bear weight on the ipsilateral limb excluding this option from treatment. In analogy to human medicine, a selective rhizotomy may reduce spasticity without inducing a paralysis of the affected muscle. During this procedure the dorsal spinal roots which communicate with the spastic muscle are transected (Arnault, 1982). This technique has already been successfully performed in three animals suffering from BSP-G (De Ley and De Moor, 1977). Further research is necessary to develop this technique for animals suffering from BSP-Q and BSP-M and to evaluate its success rate.

Conclusion

Differentiation between the three entities of spastic paresis by careful clinical examination and optional epidural injection of 0.38% procaine is imperative to perform an adequate treatment and to reliably predict its success rate. In BSP-G animals a good outcome is observed after surgical treatment. In BSP-M cases without predominant spasticity of the quadriceps femoris muscle, surgery is a possible solution, but a lower success percentage after treatment is obtained in comparison with BSP-G calves. Treatment options for BSP-Q animals are not available and a routine selective tibial neurectomy is expected to worsen the clinical signs. However, based on the findings of this study no conclusions can be drawn on response to surgical therapy for BSP-Q calves. Nevertheless, spontaneous improvement is possible in these animals.

References

- Baird J.D., Johnston K.G., Hartley W.J., 1974. Spastic paresis in Friesian calves. *Australian Veterinary Journal* 50, 239-245.
- Barvalia D.R., Patil D.B., 2003. Modified gastrocnemius tenectomy with intact deep calcaneal tendinous tissues to relieve spastic paresis in bullocks. *Veterinarski Arhiv* 73, 295-300.
- Bouckaert J.H., De Moor A., 1966. Treatment of spastic paralysis in cattle: improved denervation technique of the gastrocnemius muscle and postoperative course. *Veterinary Record* 79, 226-229.
- Budras K., Habel R.E., 2003. *Bovine Anatomy. An illustrated Text.* Schlütersche, Hannover, pp 17.
- De Ley G., De Moor A., 1977. Bovine spastic paresis: results of surgical desafferentation of the gastrocnemius muscle by means of spinal dorsal root resection. *American Journal of Veterinary Research* 38, 1899-1900.
- De Ley G., De Moor A., 1980. Bovine spastic paralysis: results of selective gamma efferent suppression with dilute procaine. *Veterinary Science Communications* 3, 289-298.
- Gadgil B.A., Agarwal S.P., Patel U.G., 1970. Spastic paresis in adult Indian cattle. *Veterinary Record* 86, 694-697.
- Gentile A., Testoni S., 2006. Inherited disorders of cattle: a selected review. *Slovenian Veterinary Research* 43, 17-29.
- Griffiths I.B., 2005. Spastic paresis in calves. *Veterinary Record* 157, 786-787.
- Hamoir J., 1922. La contracture des muscles jumeaux chez le boeuf. *Echo Vétérinaire* 51, 163-175.

- Keith J.R., 1981. Spastic paresis in beef and dairy cattle. *Veterinary Medicine & Small Animal Clinician* 76, 1043-1047.
- Ledoux J.M., 2001. Bovine spastic paresis: etiological hypotheses. *Medical Hypotheses* 57, 573-579.
- Leipold H.W., Huston K., Guffy M.M., Noordsy J.L., 1967. Spastic paresis in beef Shorthorn cattle. *Journal of the American Veterinary Medical Association* 151, 598-601.
- Touati K., Muller P., Grulke S., Peters F., Serteyn D., 2003. La parésie spastique du quadriceps fémoral: Une nouvelle entité clinique chez le veau de race Blanc Bleu Belge. *Annales de Médecine Vétérinaire* 147, 261-265.
- Vertenten G., 2006. Recente evolutie van spastische parese bij het kalf. *Het Dierenartsen Weekblad* 44, 14-17.
- Vlaminck L., De Moor A., Martens A., Steenhaut M., Gasthuys F., Desmet P., Van Branteghem L., 2000. Partial tibial neurectomy in 113 Belgian blue calves with spastic paresis. *Veterinary Record* 147, 16-19.
- Weaver A.D., 1991. Modified gastrocnemius tenectomy: a procedure to relieve spastic paresis in dairy calves. *Veterinary Medicine* 86, 1234-1239.

CHAPTER 4

Diagnosing quadriceps femoris muscle involvement in bovine spastic paresis

SECTION 4.1

Femoral nerve blocking: cadaver study

Adapted from:

De Vlaminck C., Pille F., Hauspie S., Saunders J., Van der Stede Y., Gasthuys F., Vlaminck L., 2013. Evaluation of three approaches for performing ultrasonography-guided anesthetic blockade of the femoral nerve in calves. *American Journal of Veterinary Research* 74, 750-756.

Summary

Diagnostic perineural anaesthesia of the femoral nerve might be helpful in identifying quadriceps femoris muscle involvement in complex spastic paresis cases in bovines. In the present study, three different injection approaches of the femoral nerve (ventral paravertebral, dorsal paravertebral and ilial approach) were evaluated by simulated ultrasound-guided perineural injection of methylene blue in 10 cadavers. The study was preceded by detailed anatomical and cross-sectional investigation of relevant topography in 3 cadavers, to establish the best possible injection approaches. Ultrasound image quality, the number of needle redirections required for correct needle positioning, and the resultant injection success (injection score) were recorded. The dorsal paravertebral approach yielded the best results with 80% of targeted nerves properly stained after injection. It was concluded that this technique was preferred over others for use as a diagnostic tool in the differentiation of different spastic paresis entities. Further evaluation in an in vivo setting is required to validate its clinical use.

Introduction

The typical clinical manifestation of spastic paresis in cattle consists of involuntary spastic contractions of the gastrocnemius muscle when the cattle are standing. In contrast, a variant manifestation in Belgian Blue calves is mainly characterized by quadriceps femoris muscle involvement in spasticity of the hindquarters (Touati et al., 2003). To the authors' knowledge, involvement of the quadriceps femoris muscle in spastic paresis in cattle still needs to be confirmed.

Spastic paresis of the gastrocnemius muscle or quadriceps femoris muscle in calves is differentiated through the evaluation of posture and gait. Calves with spastic paresis of the gastrocnemius muscle have spastic hyperextension of the affected hind limb in a caudal direction, whereas those in which the quadriceps femoris muscle is affected primarily have cranially directed hyperextension of the limb. In recent years, a higher than usual incidence of mixed spastic paresis involving both muscles has been observed in Belgian Blue calves. Spasticity of gastrocnemius, quadriceps femoris, and probably other muscle groups of the hind limb causes repetitive hyperextension of the affected limb. Depending on the dominant spastic muscle, this hyperextension is variably directed cranially, caudally or laterally, complicating definitive diagnosis.

Standard treatment for spastic paresis includes tenectomy or surgical denervation (selective neurectomy of the tibial nerve) of the gastrocnemius muscle bellies; however, no treatment has been described for mixed spastic paresis (De Moor et al., 1964; Pavaux et al., 1985). Selective tibial neurectomy to denervate the gastrocnemius muscle is contraindicated when the quadriceps femoris muscle is a major contributor to the spasticity. The overactivity of quadriceps femoris and gastrocnemius muscles can keep the affected limb in a neutral, although spastically hyperextended, position. When the influence of a contributing muscle is removed, as would occur with partial neurectomy of the gastrocnemius muscle, spastic contractions originating from the other contributing muscles become dominant and possibly exaggerated. This situation can cause an inability to remain standing. When the gastrocnemius muscle is the primary contributor to the spasticity, surgical intervention can ameliorate pain

and improve growth in an affected calf; however, a calf with mixed spastic paresis will continue having spastic contractions.

For the aforementioned reasons, it is important to be able to differentiate among the various types of spastic paresis. Differentiation through clinical examination alone is challenging, and findings are often inconclusive. Anaesthetic nerve blocks targeted to affect the spastic muscles can temporarily relieve signs and may aid in identification of the contributing muscle groups, and this procedure is used for that purpose in humans, such as those that have had a traumatic brain injury or stroke (Filipetti and Decq, 2003; Esquenazi, 2004; Buffenoir et al., 2005). In humans, the clinical effects of anaesthetic blocks are useful in determining appropriate therapeutic interventions such as neurolysis, botulinum toxin injection, or neurectomy (Buffenoir et al., 2005; Viel et al., 2005).

In veterinary medicine, nerve blocks are commonly performed for diagnostic purposes such as lameness examination in horses (Bassage and Ross, 2011) or for analgesic purposes before, during, and after surgical interventions (eg. paravertebral anaesthesia for laparotomy in cows) (Ivany and Muir, 2004). Given that the femoral nerve solely regulates the quadriceps femoris muscle activity, a diagnostic anaesthetic technique for blocking femoral nerve conduction might help in differentiating the muscles involved in cattle with spastic paresis. Femoral nerve blockage has been performed in dogs to provide preemptive analgesia for orthopedic procedures (Campoy et al., 2010; Echeverry et al., 2010; Echeverry et al., 2012). Comparable techniques for use in cattle have not been reported. The purpose of the study reported here was to explore various approaches for a practical ultrasonography-guided perineural injection of the femoral nerve in calve cadavers, to assess the accuracy of the techniques, and to identify the superior technique so that it might be evaluated further in live cattle.

Materials and methods

Animals

The study involved 2 phases. In the first, the topographic anatomy of the femoral nerve was evaluated in the cadavers of 2 calves (a 78-kg Belgian Blue calf [age, 6 weeks] and a 52-kg Holstein calf [age, 4 weeks]), and the cross-sectional anatomy was evaluated in an additional Holstein calf (50 kg; age, 4 weeks). All calves had been euthanized for reasons unrelated to any pathological changes involving their neuromusculoskeletal structures.

In the second phase, ultrasonography-guided approaches to perineural injection of the femoral nerve were evaluated in the cadavers of 10 healthy 4-week-old male Holstein-Friesian calves with a median body weight of 50 kg (range, 45 to 55 kg). These calves had been used in unrelated toxicological studies that required euthanasia. The protocol for both phases of the present study was approved by the Ethical Committee for Animal Research of Ghent University (EC No. 2011-057).

Topographic and cross-sectional anatomic evaluation of the femoral nerve

Topographic anatomy of the femoral nerve was reviewed with the aid of anatomy textbooks (Ashdown et al., 1996; Budras and Habel, 2003). Dissection of the 2 calf cadavers was performed within 4 hours after euthanasia. Initially, the cadavers were positioned in dorsal recumbency for dissection of the medial thigh and inguinal region. Afterwards, the calves were positioned in lateral recumbency for dissection of the lumbosacral region and the hindquarter musculature. Anatomic landmarks relevant for ultrasonography of the regions of interest were recorded, and possible approaches for needle insertion were identified.

For the cross-sectional evaluation, the spinal cord and musculature of the back of the third cadaver was removed within 2 hours after death and frozen for 48 hours at -18°C . Consecutive 2-cm-thick transverse sections were subsequently cut from L2 up to Ca2. High-resolution photographs were obtained of each side of the frozen transverse sections (Canon EOS50D, 50mm Sigma 1:2.8 macro lens, Canon, Tokyo, Japan) on a photography table (Cambo Bv, Kampen, The Netherlands). Topographic relationships of important structures were studied on the photographs for comparison with corresponding ultrasonographic images. Ultrasonography of the regions of interest was performed with a 5-MHz curved linear array transducer and ultrasonography machine (Pro Series model number 2270968, GE Medical Systems, Korea). The focus was set in accordance with the depth of the landmarks.

Ultrasonography-guided perineural injection of the femoral nerve

This portion of the study was performed immediately after calves were euthanized. All procedures were performed by the same operator (C. De Vlaminck), who had limited experience with ultrasonographically-guided injections. Each cadaver was positioned in lateral recumbency to mimic the clinical situation for anaesthetic nerve blockade. The skin overlying the predetermined injection sites was clipped and scrubbed. Relevant anatomic landmarks were first identified by means of ultrasonography.

Three ultrasonography-guided approaches to anaesthetic blockade of the femoral nerve were evaluated: dorsal paravertebral in 5 cadavers, ventral paravertebral in the remaining 5 cadavers, and ilial in all 10 cadavers. Using ultrasonographic guidance, a 90 mm 19 gauge spinal needle (Terumo Corp, Tokyo, Japan) was used to inject 5 ml of 20% methylene blue solution (Anmol Chemicals, Mumbai, India) around the nerve, with the position of the injection differing among approaches. The dorsal and ventral approaches were not combined on the ipsilateral side of the cadaver because both approaches targeted the same portion of the femoral nerve. A more distally located portion of the femoral nerve was stained during injection via the ilial approach, which allowed this technique

to be combined with either of the other 2 techniques on the ipsilateral side (Figure 5). After perineural nerve injection, the spinal and hindquarter musculature was dissected to expose the femoral nerve and verify the accuracy of dye deposition around the nerve. Forty injections were performed in 10 cadavers, including 10 via the ventral paravertebral approach, 10 via the dorsal paravertebral approach, and 20 via the ilial approach.

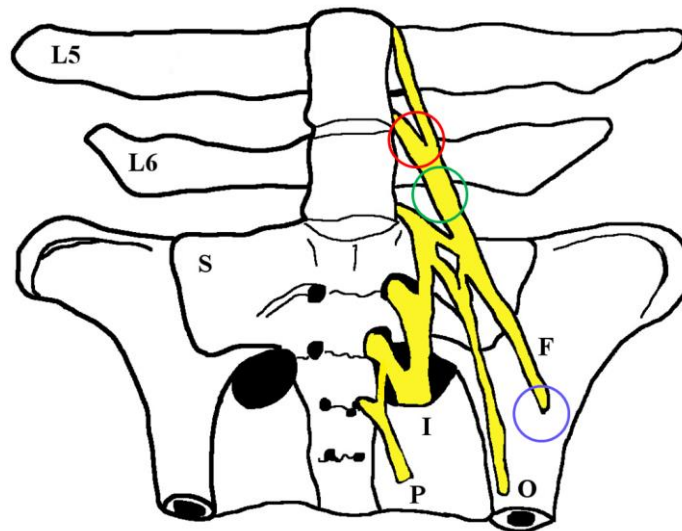


Figure 5 - Diagram of the ventral view of the lumbosacral neural plexus in a calf cadaver, showing the femoral nerve zones targeted during ultrasonography-guided perineural injection of methylene blue dye: dorsal approach (red circle), ventral approach (green circle), and ileal approach (blue circle). F = Femoral nerve. I = Sciatic nerve. O = Obturator nerve. P = Pudendal nerve. S = Sacrum. L5 = Fifth lumbar vertebra. L6 = Sixth lumbar vertebra.

Several variables were recorded to assess the outcome of each injection technique, including ultrasonographic image quality, number of needle redirections required for correct needle positioning, and injection score. Ultrasonographic image quality was scored as follows: 1 = excellent (landmarks clearly identified as 2 hyperechogenic lines [ventral paravertebral approach and dorsal paravertebral approach] or as 2 hypoechogenic spots [ilial approach]; needle clearly identified as a continuous hyperechogenic line); 2 = acceptable (landmarks or needle but not both poorly identified); 3 = poor (both landmarks and needle poorly identified).

When a needle was inaccurately positioned, it was slightly withdrawn and reinserted at a corrected angle, which was defined as a repositioning attempt. The deposition of the dye in relation to the femoral nerve was scored on a 3-point scale. An injection score of 1 was given when the outer epineurium of the nerve was stained (epineural) or 2 when the nerve was not stained but dye was found in the perineural tissues < 5 mm away from the femoral nerve (perineural). When dye was found > 5 mm away from the femoral nerve, an injection score of 3 was given (peripheral). An injection was considered successful when the outer epineurium of the nerve was stained (injection score 1).

Statistical analysis

Statistical and graphic analyses were performed with statistical software (R, version 2.14.0, R Foundation for Statistical Computing, Vienna, Austria. Available at: www.r-project.org/. Accessed Oct 31, 20 and SAS 9.3, SAS Institute Inc., Cary, NC, USA). The Kendall τ nonparametric correlation coefficient was calculated to correlate ultrasonographic image and dye injection scores. Statistical differences in both types of scores between the 3 ultrasonography-guided techniques (ventral paravertebral approach, dorsal paravertebral approach, and ilial approach) were evaluated via the Kruskal-Wallis test. The Jonckheere-Terpstra test was used to evaluate whether a trend existed in the scores obtained after injection and to test the hypothesis that a learning effect existed for the injection techniques (i.e. better injection scores for femoral nerves injected at the end of the experiment). A value of $P < 0.05$ was considered significant for all analyses.

Results

Topographic and cross-sectional anatomy of the femoral nerve

The femoral nerve was found to originate from several roots at the L4 through L6 vertebrae. These branches were surrounded by connective tissue and located near the vertebral bodies of L5 and L6, ventral to the transverse processes of these vertebrae and medial to the psoas major muscle (Figures 6 and 7). More distally, the nerve continued in a caudoventral direction toward the wing of the sacrum (ala ossis sacri) and medial to the shaft of the ilium, and passed between the tendons of the psoas minor and iliopsoas muscles, to join the external iliac artery and vein. At the level of the pecten ossis pubis, the femoral nerve gave off the saphenous nerve, which turned medially and spread sensory branches to the skin of the medial thigh and continued distally to the level of the tarsus. It also contained motor branches for several adductor muscles of the hind limb (sartorius, pectineus, and gracilis muscles). The femoral nerve continued laterally towards the femoral artery and vein. The nerve split into several branches to innervate the various parts of the quadriceps femoris muscle.

When the cadaver was positioned in dorsal recumbency, the nerve was located deep in the inguinal region, surrounded by several important blood vessels, which might become damaged when an inguinal approach is used. Therefore, three other possible routes to reach the femoral nerve by injection were proposed for study: two targeting the nerve near its origin in the paravertebral area (dorsal and ventral paravertebral approach) and one aimed at the mid-ilial shaft region (ilial approach).

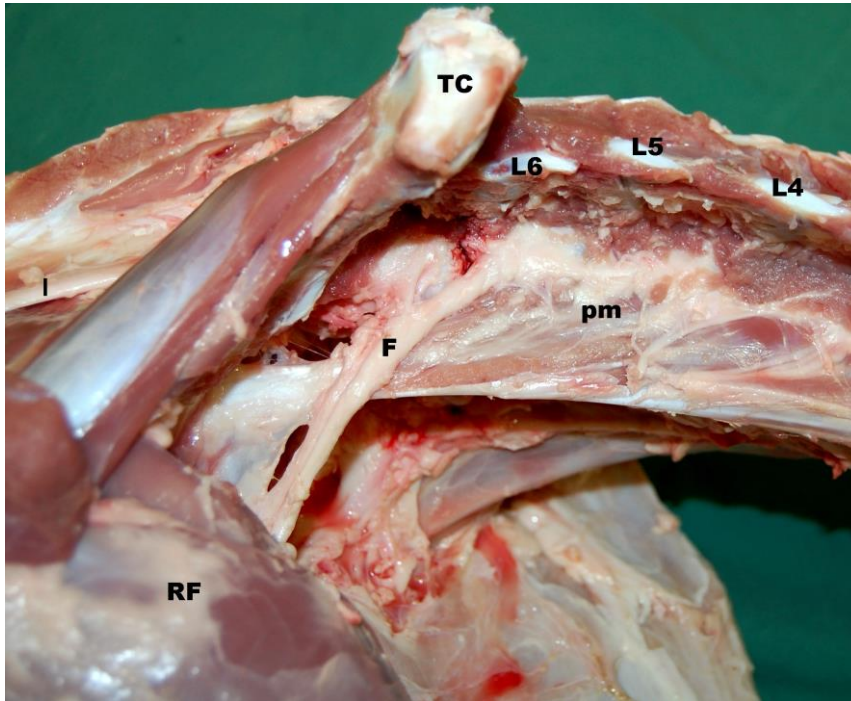


Figure 6 - Photograph showing a lateral view of the dissected lumbosacral area that reveals the origin of the femoral nerve in a calf cadaver. Cranial is to the right of the picture. F = Femoral nerve. pm = Psoas minor muscle. RF = Rectus femoris muscle. TC = Tuber coxae. I = Sciatic nerve.

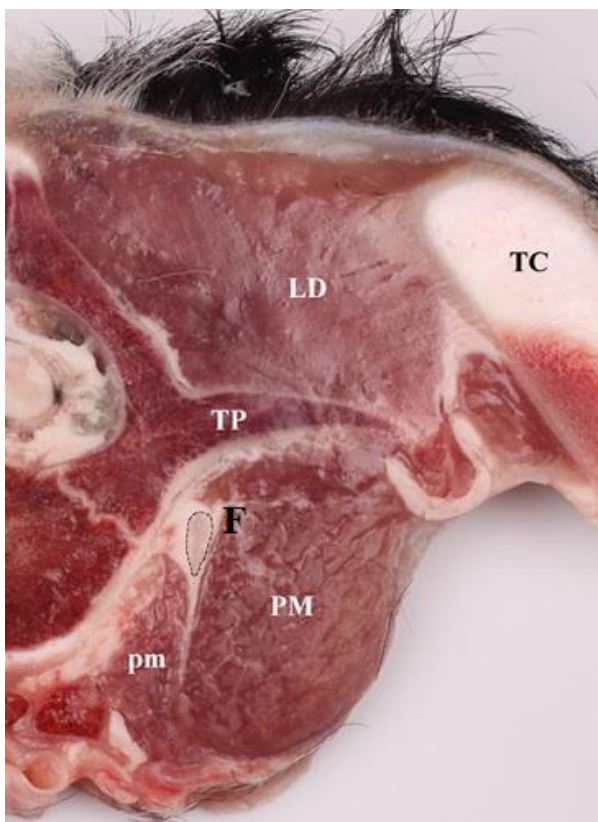


Figure 7 - Photograph showing a cranial view of a transverse section at the level of L6 vertebra in a calf cadaver. The dotted black line indicates the femoral nerve (F). LD = Longissimus dorsi muscle. pm = Psoas minor muscle. PM = Psoas major muscle. TP = Transverse process of L6. TC = Tuber coxae.

Dorsal paravertebral approach

The ultrasonography transducer was placed in an axial, transverse plane at the level of the sacrum and advanced in a cranial direction along the spinal axis to identify the space between the spinous processes of L5 and L6, which are the bony landmarks for this approach. Then the transducer was rotated 90° to a longitudinal plane and repositioned 2 to 3 cm lateral to the axis of the spinous process to visualise the space between the transverse processes of L5 and L6. A 90 mm, 19 gauge spinal needle was inserted through the skin lateral to the transducer (Figure 8) and advanced in a caudomedial direction toward the cranial border of the transverse process of L6. Once the needle tip reached a position < 1 cm lateral to the vertebral body of L6, it was further advanced for a maximum of 1 cm ventral to the transverse process, where the dye solution was injected.

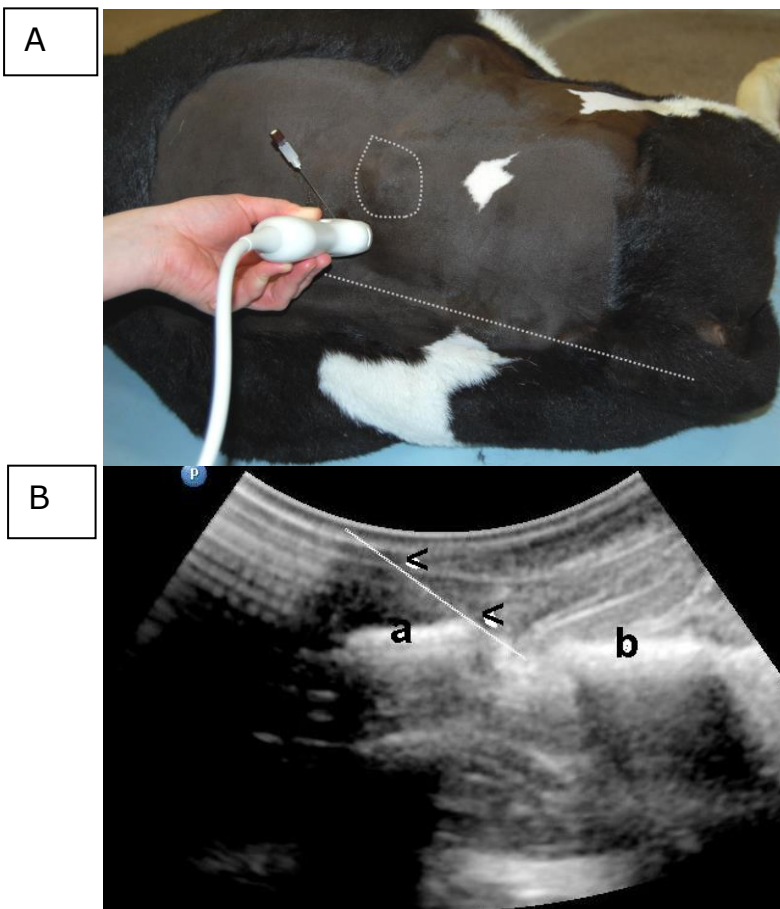


Figure 8 - Photograph (A) of the dorsal aspect of the lumbosacral area of a calf cadaver in left lateral recumbency and ultrasonographic image (B) showing the position of the needle used for a dorsal paravertebral approach for perineural injection of the femoral nerve in a calf cadaver.

In panel A, the rump of the calf is to the left and the hindquarter is to the right. The straight dotted line indicates the spinal axis of the calf, and the circular dotted line indicates the tuber coxae. In panel B, black arrowheads indicate the needle near the cranial border of the transverse process of L6; a, b: transverse process of 5th and 6th lumbar vertebrae respectively; the straight white line indicates the location of the needle.

Ventral paravertebral approach

The space between the transverse processes of the L5 and L6 vertebrae and the bony contours of these vertebrae were identified as described. With the transducer aimed at the lateral edge of the transverse processes of both vertebrae (bony landmarks), the spinal needle was inserted perpendicular to the longitudinal axis of the spinal cord, 0.5 cm ventral to the horizontal plane of the lumbar transverse processes and in the middle of the space between the transverse processes of L5 and L6 (Figure 9). As soon as the needle was identified in this region, it was oriented toward the contralateral tuber ischiadicum. During this procedure, the needle could be followed ultrasonographically until it reached the cranial border of the transverse process of L6. Further insertion made the needle disappear beneath the transverse process until it touched the body of L6. Before dye solution was injected, the needle was withdrawn a few millimeters.

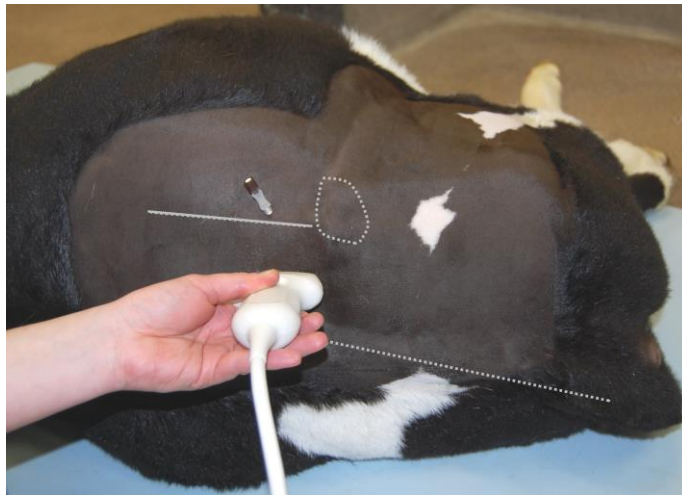


Figure 9 - Photograph of the dorsal view of the lumbosacral area of a calf cadaver in left lateral recumbency showing the position of the needle used for a ventral paravertebral approach for perineural injection of the femoral nerve. The transducer was used to identify the intertransverse process space between L5 and L6. The straight solid line indicates the lateral edge of the horizontal plane of the lumbar transverse processes.

Ilial approach

For this approach, the femoral nerve was targeted at the point where it coursed ventral to the ilial shaft and was accompanied by the femoral artery and vein, which were used as landmarks. The transducer was positioned 3 to 4 cm caudoventral to the tuber coxae, with the longitudinal axis of the transducer in 45° cranioventral – caudodorsal direction and perpendicular to the skin. Consequently, it was moved in a caudoventral direction until both blood vessels were identified as 2 hypoechogenic spots (Figure 10). The spinal needle was inserted cranial to the transducer and oriented toward the blood vessels. Once the tip of the needle was identified near this location, the dye solution was injected.

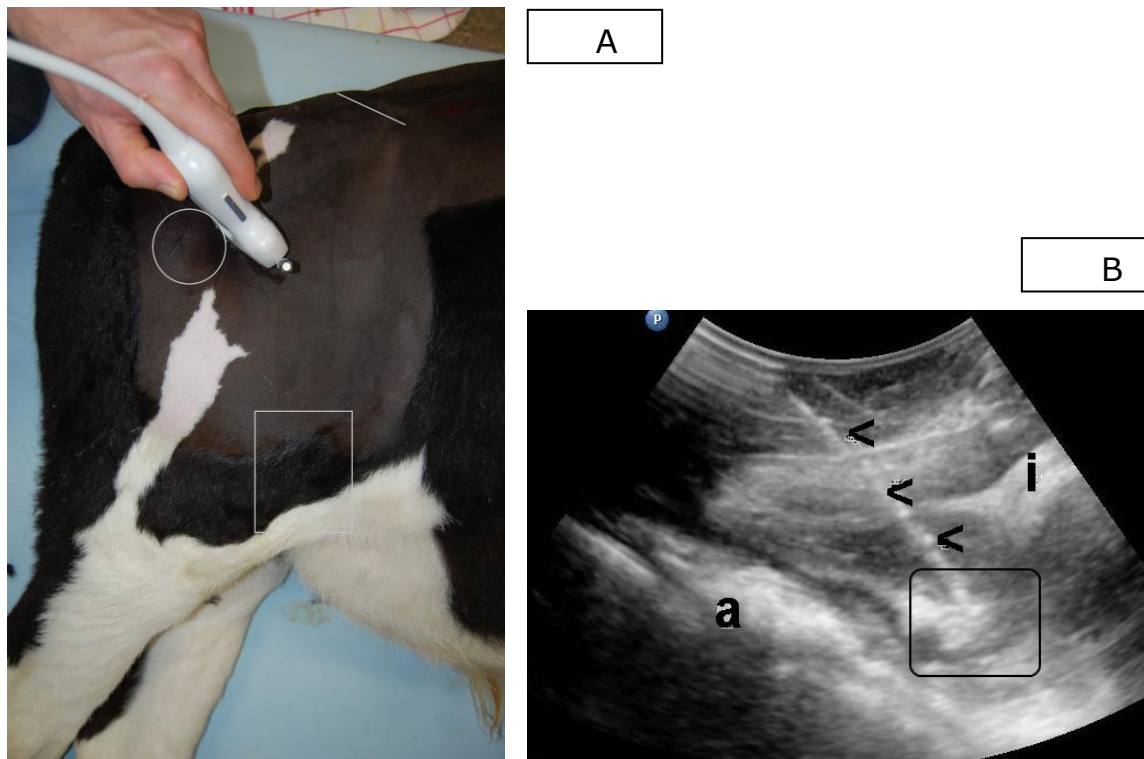


Figure 10 - Photograph (A) of the lateral view of the right gluteal region of a calf cadaver in left lateral recumbency and ultrasonographic image (B) showing the position of the needle used for an ilial approach for perineural injection of the femoral nerve in a calf cadaver. Injection was not yet performed. In panel A, the tuber coxae (white line), the location of the coxofemoral joint (circle), and the femorotibial joint of the calf (rectangle) are indicated. In panel B, the femoral artery and vein were used as landmarks as indicated (rectangle). Notice the needle near the neurovascular bundle (arrowheads). a = Abdomen. i = ilial shaft.

Simulated anaesthetic blockade of the femoral nerve

A significant ($P < 0.05$) correlation was found between the injection score and ultrasonographic image score for the ventral paravertebral approach ($\tau = 0.66$) and the ilial approach ($\tau = 0.62$) but not for the dorsal paravertebral approach ($\tau = 0.40$; Figure 11). The median number of times the needle required repositioning in the dorsal approach was 5 (range, 2 to 11). For the ventral and ilial approaches, this number was 2.5 (range, 1 to 9) and 4.5 (range, 1 to 10), respectively. The femoral nerve was stained in 8 of 10 dorsal paravertebral injections, in 5 of 10 cases by the ventral paravertebral approach, and in 8 of 20 for the ilial approach. The proportion of injections that achieved an injection score of 1 was highest in the dorsal paravertebral approach, compared with the other approaches. However, these proportions were not significantly ($P = 0.53$) different. No significant differences in injection scores ($P = 0.13$) nor in ultrasonography scores ($P = 0.65$) were observed between the different approaches. Although the injection scores improved when more injections were performed for each approach, this trend was not significant ($P > 0.10$).

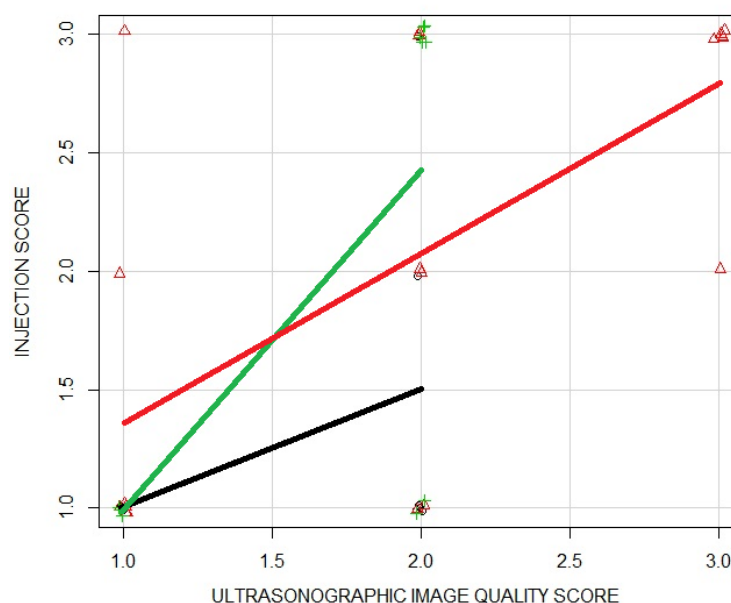


Figure 11 - Correlation between injection score and ultrasonographic image quality score for dorsal paravertebral (black), ventral paravertebral (green), and ilial (red) approaches to ultrasonography-guided perineural injection of the femoral nerve in calf cadavers ($n = 10$). Image quality was scored as follows: 1 = excellent, 2 = acceptable, and 3 = poor. Injection scoring was performed as follows: 1 = epineural, 2 = perineural, and 3 = peripheral.

Discussion

This preliminary cadaver study showed that simulated ultrasonography-guided perineural injection of a dye adjacent to the femoral nerve in calves is possible. The success rates of the 3 techniques used could be considered equally efficient for performance of femoral nerve blocks in cadavers.

Ultrasonography is the most appropriate imaging technique to guide perineural injection of peripheral nerves. It allows continuous visibility of the needle tip, which increases the safety of the technique by avoiding inadvertent intravenous or intraneural injection (Tagliafico et al., 2010; Tsui and Suresh, 2010). The success rate of peripheral nerve blocks performed with ultrasonographic guidance is considered superior to success rates for techniques that do not allow structures to be seen (Mahler and Adogwa, 2008). Ultrasonographic guidance also allows identification of bony landmarks, which is important for correct needle positioning when the injection target is specific or located deep to anatomic areas or structures. This characteristic is of major importance in well-muscled beef cattle, in which bony landmarks are not as readily palpable as they are in leaner breeds. Holstein calves were used in the present study mainly because of economic considerations and availability. Their body conformation facilitated the application of the approaches by a moderately experienced operator.

In humans and dogs, a femoral nerve block is performed through a medial, inguinal region approach (Campoy et al., 2010; Echeverry et al., 2010; Mahler and Adogwa, 2008). The topographic and cross-sectional anatomic evaluation in the present study showed that an inguinal approach to perineural nerve injection was highly impractical, mainly because of the large muscle volume often encountered in beef calves and the high risk of inadvertent blood vessel damage. Clinical application of this approach would also require deep sedation of a calf to allow a safe approach, followed by sedative reversal for subsequent gait evaluation, which would further complicate the procedure. Lateral approaches were deemed more efficient than an inguinal approach because of the more superficial location of the femoral nerve and the proximity of specific anatomic

landmarks such as the transverse processes of the caudal lumbar vertebrae, the tuber coxae, and important adjacent vascular structures. However, in the paravertebral approaches, the femoral nerve was not directly visible because of the nerve's location close to the lumbar vertebral column. In the dorsal paravertebral approach, the needle tip could be accurately advanced to the cranial border of the transverse process of L6. In the ventral paravertebral approach, ultrasonography was only useful to identify the correct position for needle insertion. Further insertion was performed blindly and guided by external characteristics such as the contralateral tuber ischiadicum. This complication might explain the lower success rate associated with the ventral technique. The ventral paravertebral approach targets a slightly more caudal area of the femoral nerve than does the dorsal paravertebral approach, which might overlap with the origin of the obturator nerve in some calves. Deposition of local anaesthetic in this region could cause paralysis of quadriceps femoris and adductor muscles, which might complicate clinical evaluation of the nerve block. For this reason, the least amount of anaesthetic should be used when the ventral approach is used.

The main difficulty encountered with the ilial approach was the correct identification of the femoral artery and vein. In several situations, these vascular structures were not clearly outlined on the ultrasonographic images, mainly because of the absence of blood flow in the cadavers, which precluded the use of Doppler techniques to enhance their detection. The inability to clearly see these landmarks in cadavers may have contributed to a lower image quality score for the ilial versus other approaches. Use of the ilial approach in live calves might provide a better visibility of the landmarks and therefore better results than those obtained in this cadaver study. The ilial approach enabled nerve identification and allowed needle guidance to the perineural level. Furthermore, injection of a dye solution could be seen as it spread along the neural and vascular structures. However, a disadvantage of the ilial approach with Doppler techniques is that it would require the use of more sophisticated equipment, increasing the financial burden of the procedure.

Conclusion

We considered the dorsal paravertebral approach to be the most user-friendly of the 3 techniques evaluated and believe that moderate experience in ultrasonography would be sufficient to obtain a high success rate for staining the targeted nerve. No sophisticated equipment was required, and the portion of the injection path that could not be seen was small. However, the small number of calves used in the present study is a limitation to the study. Clinical application of these ultrasonographic approaches in healthy cattle would be essential for confirming the suitability of the described perineural injection techniques. From a clinical perspective, the potential of the dorsal paravertebral technique to enable identification of quadriceps femoris muscle involvement in the spastic paresis syndrome warrants further investigation.

References

- Ashdown R.R., Done S.H., Barnett S.W., 1996. Color Atlas of Veterinary Anatomy: The Ruminants v.1. Mosby-Wolfe, Spain, pp 248.
- Bassage L.H., Ross M.W., 2011. Diagnostic Analgesia. In: Ross, M.W., Dyson, S.J. (Eds.), Diagnosis and management of lameness in the horse. Elsevier Saunders, Missouri, pp. 100-135.
- Budras K., Habel R.E., 2003. Bovine Anatomy. An illustrated Text. Schlütersche, Hannover, pp 138.
- Buffenoir K., Decq P., Lefaucheur J., 2005. Interest of peripheral anesthetic blocks as a diagnosis and prognosis tool in patients with spastic equinus foot : A clinical and electrophysiological study of the effects of block of nerve branches to the triceps surae muscle. *Clinical Neurophysiology* 116, 1596-1600.
- Campoy L., Bezuidenhout A.J., Gleed R.D., Martin-Flores M., Raw R.M., Santare C.L., Jay A.R., Wang A.L., 2010. Ultrasound-guided approach for axillary brachial plexus, femoral nerve, and sciatic nerve blocks in dogs. *Veterinary Anaesthesia and Analgesia* 37, 144-153.
- De Moor A., Bouckaert J.H., Top W., 1964. Chirurgische behandeling van spastische parese bij het rund door denervatie van de m. gastrocnemius. *Vlaams Diergeneeskundig Tijdschrift* 33, 1-18.
- Echeverry D.F., Gil F., Laredo F., Ayala M.D., Belda E., Soler M., Agut A., 2010. Ultrasound-guided block of the sciatic and femoral nerves in dogs: a descriptive study. *The Veterinary Journal* 186, 210-215.
- Echeverry D.F., Laredo F.G., Gil F., Belda E., Soler M., Agut A., 2012. Ventral ultrasound-guided suprainguinal approach to block the femoral nerve in the dog. *The Veterinary Journal* 192, 333-337.
- Esquenazi A., 2004. Evaluation and management of spastic gait in patients with traumatic brain injury. *Journal of Head Trauma Rehabilitation* 19, 109-118.

Femoral nerve blocking: cadaver study

- Filipetti P., Decq P., 2003. Interest of anesthetic blocs for assessment of the spastic patient. A series of 815 motor blocks. *Neurochirurgie* 49, 226-238.
- Ivany J.M., Muir W.W., 2004. Farm animal anesthesia. In: Fubini S.L., Ducharme N.G. (Eds.), *Farm Animal Surgery*. Saunders, Missouri, pp 97-112.
- Mahler S.P., Adogwa A.O., 2008. Anatomical and experimental studies of brachial plexus, sciatic, and femoral nerve-location using peripheral nerve stimulation in the dog. *Veterinary Anaesthesia and Analgesia* 35, 80-89.
- Pavaux C. L., Sautet J., Lignereux Y., 1985. Anatomie du muscle gastrocnémien des bovins appliquée à la cure chirurgicale de la parésie spastique. *Vlaams Diergeneeskundig Tijdschrift* 54, 296-312.
- Tagliafico A., Bodner G., Rosenberg I., Palmieri F., Garello I., Altafini L., Martinoli C., 2010. Peripheral nerves: ultrasound-guided interventional procedures. *Seminars in musculoskeletal radiology* 14, 559-566.
- Touati K., Muller P.H., Grulke S., Peters F., Serteyn D., 2003. La parésie spastique du quadriceps fémoral : Une nouvelle entité clinique chez le veau de race Blanc Bleu Belge. *Annuelle Médecine Vétérinaire* 147, 261-265.
- Tsui B., Suresh S., 2010. Ultrasound imaging for regional anesthesia in infants, children and adolescents: a review of current literature and its application in the practice of extremity and trunk blocks. *Anesthesiology* 112, 473-492.
- Viel E., Pellas F., Ripart J., Pélissier J., Eledjam J.-J., 2005. Spasticité : intérêt du testing par anesthésie locorégionale et blocs thérapeutiques. *Peripheral neurolytic blocks and spasticity. Annales Françaises d'Anesthésie et de Réanimation* 24, 667-672.

SECTION 4.2

Femoral nerve blocking: clinical trial

Adapted from:

De Vlaminck C., Vlaminck L., Hauspie S., Saunders J., Gasthuys F., 2013.
Ultrasound-guided femoral nerve block as a diagnostic aid in demonstrating quadriceps involvement in bovine spastic paresis. *The Veterinary Journal* 196, 451-455.

Summary

The aim of this study was to evaluate the clinical effects of a femoral nerve block via a dorsal paralumbar injection in healthy calves and in calves suffering from spastic paresis. Based on bony landmarks and using ultrasound guidance, the femoral nerves of eight healthy calves were blocked bilaterally with a 4% procaine solution containing blue dye. In 11/16 nerve blocks, paralysis of the quadriceps femoris muscle was obtained after dorsal paralumbar injection. Paralysis was total in 8/16 cases. The injection site was confirmed by post mortem dissection, and in 12/16 cases, the blue dye was found < 2 mm from the nerve. Clinical use of the technique was then demonstrated in two cases of atypical bovine spastic paresis. In such calves an objective diagnostic tool is required to identify those calves which are suitable for selective tibial neurectomy. The femoral nerve block used in this study has the potential to be such a method and can be used to establish the involvement of the quadriceps femoris muscle in calves suffering from the quadriceps or mixed presentation forms of spastic paresis.

Introduction

Bovine spastic paresis (BSP) is a long-standing developmental neuromuscular disorder (Götze, 1932). An overactive stretch reflex, primarily involving the gastrocnemius muscle (BSP-G), causes repeated spastic hyperextension of the affected hind limb of the standing animal in a caudal direction (De Ley and De Moor, 1977). However, specific quadriceps femoris muscle involvement (BSP-Q) has been reported as a new pathologic entity in this syndrome (Touati et al., 2003). Differentiation between BSP-G and BSP-Q affected calves is based on posture or gait analysis of symptoms. BSP-Q calves demonstrate repeated spastic hyperextension of one or both hind limbs in cranial direction contrary to BSP-G. However, to the authors' knowledge, the involvement of the quadriceps femoris muscle in BSP-Q has never been confirmed. Furthermore, in recent years the authors have even been confronted with increasing numbers of mixed presentations of bovine spastic paresis (BSP-M) in double-muscled Belgian Blue calves. Depending on the dominant spastic muscles, hyperextension of the affected limb can be directed cranially, caudally or laterally complicating diagnosis.

Treatment of BSP-G principally includes tenectomy or selective tibial neurectomy (Pavaux et al., 1985; Vlaminck et al., 2000). However, tibial neurectomy applied to BSP-Q or BSP-M can aggravate symptoms, e.g. it can result in exaggerated cranial hyperextension of the hind limb which can prevent normal movement (De Vlaminck et al., 2013). To the authors' knowledge, no therapy has been described for these atypical spastic paresis cases, but they need to be clearly distinguished from BSP-G in order to avoid surgery on unsuitable patients.

BSP-G and BSP-Q can be distinguished based on clinical observation. However, some animals show clinically obvious quadriceps femoris muscle spasticity, alongside overlooked spasticity of other muscles. Epidural injection of a 0.38% procaine solution can resolve BSP-G symptoms (De Ley and De Moor, 1980) but does not resolve the symptoms associated with BSP-Q and BSP-M (Vertenten,

2006). Significantly increased gastrocnemius muscle activity has been reported in electromyography studies on BSP-G calves but voluntary muscle activity might falsely contribute to the electromyographic read-out and hinder objective identification of the involved muscles (Denniston et al., 1968; Bijleveld and Hartman, 1976). As quadriceps femoris muscle activity is solely controlled by the femoral nerve (Budras and Habel, 2003), diagnostic anaesthesia might be a better tool to identify which muscles are involved in spastic paresis. Nerve blocks have been routinely performed in man to identify muscles contributing to spasticity e.g. in patients following traumatic brain injury or stroke (Filippetti and Decq, 2003; Esquenazi, 2004; Buffenoir et al., 2005). Observing post-injection posture and gait in calves could help in decision making about treatment options.

The aim of the present study was to evaluate a technique for perineural injection of the femoral nerve in healthy calves. It was hypothesized that the technique could reliably induce femoral nerve paralysis in a safe and reversible manner. Implementation of the technique was then further evaluated in two calves, viz., one with BSP-Q, and one with BSP-M.

Materials and methods

In vivo femoral nerve block in healthy calves

The study design was approved by the Ethical Committee for Animal Research of Ghent University (EC 2011-079). Eight healthy male Holstein Friesian calves aged 4 weeks, with a median weight of 50 kg (range 47-53 kg) were used.

PROCEDURE

All procedures were performed by the same operator (C. De Vlamynck). Calves were positioned in lateral recumbency by applying gentle physical restraint without sedatives. The back hair was clipped and the skin washed and disinfected with alcohol. An ultrasound-guided dorsal paralumbar approach was used, as this had been shown in a pilot study in cadavers to be the most reliable technique for perineural injection (see Section 4.1). The transverse processes of L5 and L6 were identified ultrasonographically using a 5 MHz curved linear array transducer (Pro Series model number 2270968, GE Medical Systems, Korea). The transducer was then placed in a transverse plane at the level of the sacrum and advanced in a cranial direction along the spinal axis to identify the space between the spinous processes of L5 and L6. The transducer was then rotated 90° to a longitudinal plane and repositioned 2-3 cm lateral to the spinous process axis to visualize the intertransverse process area. A 90 mm 19 gauge spinal needle was inserted 1 cm through the skin, lateral to the transducer and advanced in a caudomedial direction towards the cranial border of the transverse process of L6. Once the needle tip reached a position less than 1 cm lateral to the vertebral body of L6, it was further advanced over a distance of maximum 1 cm under the transverse process. A aqueous solution including 1 ml of methylene blue mixed with 4 ml of procaine 4% (VMD, Arendonk) was then injected.

Ultrasound image quality was scored according to the criteria outlined in Table 4. The number of attempts to advance the needle to the correct position was recorded. Following injection, calves were placed in a rubber padded box. Twenty minutes later, sensitivity of the ipsilateral medial thigh was tested at the level of the femur by stimulating the skin with a needle and comparing the animal's

reaction to the same procedure on the contralateral non-treated limb. A positive result was recorded if the animal showed no reaction to stimulation. Any reaction was recorded as a negative result. Repeat injections were not performed in these cases. After this, the calves' posture and gait were observed and the success of the nerve block was categorized using a paralysis score (Table 5).

Table 4 - Criteria used for evaluation of ultrasound image quality during femoral nerve blocking in calves.

Image quality	Characteristics of posture and gait
Excellent	Bony landmarks clearly identified Needle clearly identified
Good	Bony landmarks clearly identified Needle fairly visualized
Poor	Bony landmarks clearly identified Needle poorly visualized

Table 5 - Paralysis score following femoral nerve block in calves.

Paralysis score	Characteristics of posture and gait
1 Total paralysis	Not fully weight bearing with semi-flexion of the fetlock joint at rest Animal is unable to fix the femorotibial joint; hind limb is dragged at walk
2 Partial paralysis	Normal posture at rest Incomplete extension of the femorotibial joint at walk
3 No paralysis	Normal posture at rest Normal gait at walk

Nerve blocks were performed bilaterally. The second injection, in the contralateral limb was done 60 min following the first injection (if that was successful), after complete disappearance of paralysis symptoms and the return of sensitivity of the medial thigh, or 20 min after the first injection if the first block was unsuccessful. Following recording of all data, calves were euthanized using IV xylazine (0.16 mg/kg, Xyl-M 2%, VMD) and embutramide (20 mg/kg, T61, Intervet). The spinal musculature was carefully dissected to expose the femoral nerve and verify the accuracy of the injection using an injection score (Table 6).

Table 6 - Injection score based on dye localization in relation to the femoral nerve.

Injection score	Identification of blue dye	
1	Epineural	Outer epineurium of the nerve stained
2	Perineural	Nerve not stained, dye in perineural tissues (connective and muscle tissue) <5 mm from femoral nerve
3	Peripheral	Dye >5 mm from femoral nerve

STATISTICAL ANALYSIS

Statistical analysis was performed using SPSS Statistics 19 (IBM) to evaluate the association between the ultrasonographic image quality, paralysis score and injection score using Fisher's exact test.

Clinical application of femoral nerve block in spastic paresis cases

The same nerve block was performed in two clinical cases referred for bilateral spastic paresis where treatment with a selective tibial neurectomy had been proposed. The injection was performed on the most prominently affected limb and the resulting relief of symptoms was used to guide the treatment decision.

Results

In vivo femoral nerve block in healthy calves

The results of the 16 nerve blocks are summarized in Table 7. Ultrasonographic image quality was good in 3/16 cases, acceptable in 9/16 and poor in 4/16. The ultrasound quality was not significantly associated with paralysis score ($P=0.105$), injection score ($P=0.145$) or skin sensitivity ($P=0.077$). The median number of attempts to obtain accurate needle placement was 4 (range 2 – 11). Four calves reacted while the needle was inserted through the skin. This did not affect the accuracy of the nerve block as the calves calmed down once the needle was inserted.

Table 7 - Summarized results analyzing US-guided anaesthesia of the femoral nerve in both hind limbs of 8 calves.

Calf number	Image quality	Repositioning attempts	Skin sensitivity	Paralysis score	Injection score	Remarks
1	poor	4	pos	2	2	
	poor	3	pos	1	1	
2	acceptable	10	neg	3	3	
	good	1	pos	1	1	
3	acceptable	1	neg	3	3	bilateral paralysis
	acceptable	5	pos	1	1	
4	acceptable	5	neg	3	3	
	acceptable	3	pos	1	1	
5	acceptable	6	neg	3	3	bilateral paralysis
	poor	4	pos	1	1	
6	acceptable	3	pos	2	2	
	poor	2	pos	1	1	
7	good	2	pos	1	1	
	good	3	pos	1	1	
8	acceptable	2	pos	2	2	
	acceptable	6	neg	3	2	

Pos, positive; neg, negative.

Loss of skin sensitivity of the medial thigh was recorded in 11/16 cases. Complete and partial femoral nerve paralysis were seen in 8/16 and 3/16 cases, respectively. All calves presenting total or partial paralysis demonstrated complete loss of skin sensitivity ($P < 0.001$). Two calves which showed no paralysis 20 min after the injection, developed bilateral femoral paralysis following the second nerve block on the contralateral limb. Epineural and perineural injections were recorded in 8/16 and 4/16 cases, respectively. Injection and paralysis score were significantly associated with each other ($p < 0,001$).

Clinical application of US-guided diagnostic anaesthesia of the femoral nerve

CASE 1

A 3-week-old male Belgian Blue calf (50 kg) was referred for selective tibial neurectomy. Progressive spastic contractions in both hind limbs were observed since 2 weeks. While standing, the calf continuously shifted weight from one hind limb to the other. Both hind limbs were hyperextended and, when not weight-bearing, were moved cranially in a pendulum-like fashion (Figure 12 a). The animal was only able to stand for a short period of time. Palpation of the hind limb muscles during standing revealed bilateral quadriceps femoris muscle spasms which resulted in a tin soldier's gait at walk. A tentative diagnosis of bilateral BSP-Q was made.



Figure 12 a - Belgian Blue calf presented with bilateral quadriceps femoris muscles related spastic paresis (case 1). Both hind limbs were hyperextended due to spasticity of the quadriceps femoris muscles.

A femoral nerve block was performed on the right hind limb using 5 ml of procaine 4%. Twenty minutes later, a paralysis score of 1 and complete loss of skin sensitivity in the medial thigh were observed (Figure 12 b) and resulted in absence of spastic movements in the treated limb. Spasticity of the left limb became less obvious because the calf was forced to primarily bear weight on that limb. The animal was able to remain standing for > 30 min. A presumptive diagnosis of bilateral BSP-Q was made, a poor prognosis was given and the calf was euthanized.



Figure 12 b - The same calf confined in a rubber padded box (case 1), 20 min following anaesthesia of the right femoral nerve. The common calcanean tendon is relaxed. When walking, the toes were dragged over the ground.

CASE 2

A 5-month-old female Belgian Blue calf (128 kg) was presented for selective tibial neurectomy to treat spastic paresis in both hind limbs. When standing, the calf was only weight-bearing on the right hind limb. The toe of the left hind limb failed to touch the ground; instead the limb was continuously held in an abducted position and spastically moved in cranial and caudal direction. Palpation of the common calcanean tendon revealed increased tension due to spasm of the gastrocnemius muscle heads. Palpation of the left quadriceps femoris muscle also revealed increased muscle tone. Spasticity in the right hind limb was difficult to be judged due to the constant weight bearing. At walk, both hind limbs were hyperextended and showed continuous lateral swinging-pendulum movements, which caused the animal great difficulty in maintaining its balance.

A left femoral nerve block produced, within 20 min, a paralysis score of 1 and complete loss of skin sensitivity in the medial thigh. The calf was able to bear weight on all four feet. Slight loss of adductor activity was observed. While walking, pendulum movements of the left hind limb were absent. However, continuous spasticity of other muscle groups including the gastrocnemius muscle continued to hamper normal ambulation. A presumptive diagnosis of bilateral BSP-M was made and selective tibial neurectomy was declined based on the probability that symptoms would worsen following surgery. The calf returned home but was euthanized a few weeks later because of increased ambulatory problems.

Discussion

The present study showed that ultrasound-guided blocking of the femoral nerve was feasible in healthy calves and gave good reproducible results. This study also confirmed that the quadriceps femoris muscle can be involved in BSP-Q, and that a femoral nerve block can be useful to differentiate BSP-Q and BSP-M from BSP-G.

The nerve block procedure in the present study was performed on non-sedated calves. Gentle physical restraint was sufficient to keep all animals in lateral recumbency. In heavier animals the use of a sedative might be indicated. Alpha-2 agonists are known to decrease peripheral sensitivity and may provoke recumbency in bovines, thus interfering with proper analysis of nerve block results. However, these unwanted effects may be counteracted by administering an antidote (Tranquilli et al., 2007). Local anaesthesia of the skin by subcutaneous injection of a small volume of local anaesthetic solution with a small sized needle is required to prevent the adverse reactions seen in four animals in this study while inserting the spinal needle.

Paravertebral visualization of the femoral nerve is not possible using ultrasonography because the nerve is hidden underneath the transverse processes. The technique described in this study uses easily identifiable bony landmarks to guide needle insertion; as a consequence image quality had no influence on the accuracy with which local anaesthetic was applied, illustrating the low complexity of the procedure. A complicating factor might be the increased muscle volume encountered in older and heavily muscled animals, which might necessitate the use of longer needles to accurately reach the target area. However, no problems were encountered in the 128 kg calf of double-muscled Belgian Blue origin.

Successful epineural injection of the anaesthetic solution resulted in complete reversible paralysis of the femoral nerve; the closer the anaesthetic was deposited to the nerve, the more complete the ensuing paralysis. Two calves that initially showed no response within 20 min of the first nerve block,

subsequently developed bilateral paralysis following the second contralateral nerve block. Dissection showed peripheral deposition of the local anaesthetic solution at the first block in both animals resulting in a long diffusion time to reach the femoral nerve. We suggest that at least 30 min should elapse before evaluating post injection posture changes.

Although only 69% of blocks were successful, the accuracy of the nerve block can be checked by the loss of skin sensitivity on the medial thigh, as all calves in this study presenting total or partial paralysis also had complete loss of skin sensitivity in this region. Therefore, nerve block failure can be easily identified and false assessment of the involvement of the quadriceps femoris muscle in the symptoms avoided. Furthermore, if the animal shows reaction to stimulation, repeat injections can be performed.

A draw-back of the described technique is the possible interference with obturator nerve function as was suspected in case 2. Cadaver studies have shown a relative close proximity of the obturator nerve origin near the targeted area (De Vlaminck et al., 2013). Accurate deposition of a small volume of anaesthetic solution might overcome this complication. Injection accuracy might be enhanced by the use of a peripheral nerve stimulator in which the insulated needle is used both to stimulate the targeted nerve and to deliver the local anaesthetic (Campoy et al., 2010; Echeverry et al., 2010; Viel et al., 2005). However this technique requires general anaesthesia and is thus impractical for a diagnostic technique which requires a conscious and ambulatory patient. Moreover, Tran de et al. (2008) found no substantial benefits of using stimulating perineural catheters on the efficiency of a nerve block in comparison to blinded or US-guided techniques. Nevertheless, further validation of the technique is necessary before widespread use in a clinical setting allowing objective evaluation in 100% of cases.

Diagnostic anaesthesia of the femoral nerve may help to identify quadriceps femoris muscle involvement in spastic paresis of the hind limbs. Especially in mixed types of spasticity, clinical examination often remains inconclusive. Some animals show clinically obvious quadriceps femoris or gastrocnemius muscle spasticity while other muscles are also involved but are overlooked. Their involvement will appear after successful perineural injection which then allows

objective evaluation of what further treatment is possible. When the quadriceps femoris muscle contributes significantly to the spasticity, selective tibial neurectomy to denervate the gastrocnemius muscle is contraindicated, as removal of the overactivity of the gastrocnemius muscles alone leads to exaggerated hyperextension of the femorotibial joint and, often, inability to standing for more than a few seconds. However, when there is a substantial involvement of the gastrocnemius muscle, surgery can reduce pain and increase growth, so surgery can be justified on an economic basis even though the calf will keep on having spastic contractions.

Conclusion

The present study demonstrated the potential value of a unilateral femoral nerve block as a diagnostic tool in the differentiation between and the management of BSP-Q or BSP-M in calves.

References

- Bijleveld K., Hartman W., 1976. Electromyographic studies in calves with spastic paresis. *Tijdschrift voor diergeneeskunde* 101, 805-808.
- Budras K.D., Habel R.E., 2003. *Bovine anatomy. An illustrated text.* 1ed., Schlütersche GmbH & Co. KG, Hannover, Germany, p 101.
- Buffenoir K., Decq P., Lefaucheur J., 2005. Interest of peripheral anesthetic blocks as a diagnosis and prognosis tool in patients with spastic equinus foot : A clinical and electrophysiological study of the effects of block of nerve branches to the triceps surae muscle. *Clinical Neurophysiology* 116, 1596-1600.
- Campoy L., Bezuidenhout A.J., Gleed R.D., Martin-Flores M., Raw R.M., Santare C.L., Jay A.R., Wang A.L., 2010. Ultrasound-guided approach for axillary brachial plexus, femoral nerve, and sciatic nerve blocks in dogs. *Veterinary Anaesthesia and Analgesia* 37, 144-153.
- De Ley A., De Moor A., 1977. Bovine spastic paralysis: Results of surgical desafferentation of the gastrocnemius muscle by means of spinal dorsal root resection. *American Journal of Veterinary Research* 38, 1899-1900.
- De Ley A., De Moor A., 1980. Bovine spastic paralysis: Results of selective γ -efferent suppression with dilute procaine. *Veterinary Science Communications* 3, 289-298.
- Denniston J.C., Shive R.J., Friedli U., Boucher W.B., 1968. Spastic Paresis Syndrome in Calves. *Journal of the American Veterinary Medical Association* 152, 1138-1148.
- De Vlaminck C., Pille F., Hauspie S., Saunders J., Van der Stede Y., Gasthuys F., Vlaminck L. (2013). Evaluation of three approaches for performing ultrasonography-guided anesthetic blockade of the femoral nerve in calves. *American Journal of Veterinary Research* 74, 750-756.

- Echeverry D.F., Gil F., Laredo F., Ayala M.D., Belda E., Soler M., Agut A., 2010. Ultrasound-guided block of the sciatic and femoral nerves in dogs: a descriptive study. *Veterinary Journal* 186, 210-215.
- Esquenazi A., 2004. Evaluation and management of spastic gait in patients with traumatic brain injury. *Journal of Head Trauma Rehabilitation* 19, 109-118.
- Filipetti P., Decq P., 2003. Interest of anesthetic blocs for assessment of the spastic patient. A series of 815 motor blocks. *Neurochirurgie* 49, 226-238.
- Gadgil B.A., Agarwals P., Patel U.G., 1970. Spastic paresis in adult Indian cattle. *The Veterinary Record* 86, 694-697.
- Götze R., 1932. Spastische Parese der hinteren Extremität bei Kalber und Jungrinder. *Deutsche Tierärztliche Wochenschrift* 40, 197-200.
- Pavaux C. L., Sautet J., Lignereux Y., 1985. Anatomie du muscle gastrocnémien des bovins appliquée a la cure chirurgicale de la parésie spastique. *Vlaams Diergeneeskundig Tijdschrift* 54, 296-312.
- Touati K., Muller P.H., Grulke S., Peters F., Sertheyn D., 2003. La parésie spastique du quadriceps fémoral: Une nouvelle entité clinique chez le veau de race Blanc Bleu Belge. *Annuelle Médecine Vétérinaire* 147, 261-265.
- Tran de GH., Munoz L., Russo G., Finlayson R.J., 2008. Ultrasonography and stimulating perineural catheters for nerve blocks : a review of the evidence. *Canadian Journal of Anaesthesia* 55, 447-457.
- Tranquilli W.J., Thurmon J.C., Grimm K.A., 2007. *Lumb & Jones' Veterinary Anesthesia and Analgesia*, 4ed., Blackwell Publishing, Oxford, pp 1048.
- Vertenten G., 2006. Recente evolutie van spastische parese bij het kalf. *Het Dierenartsen Weekblad* 44, 14-17.
- Viel E., Pellas F., Ripart J., Péliissier J., Eledjam J.-J., 2005. Spasticité : intérêt du testing par anesthésie locorégionale et blocs thérapeutiques. *Peripheral neurolytic blocks and spasticity. Annales Françaises d'Anesthésie et de Réanimation* 24, 667-672.

Vlaminck L., De Moor A., Martens A., Steenhaut M., Gasthuys F., Desmet P., Van Branteghem L., 2000. Partial tibial neurectomy in 113 Belgian blue calves with spastic paresis. *Veterinary Record* 147, 16-19.

CHAPTER 5

Surgical management of atypical presentations of bovine spastic paresis

SECTION 5.1

Recording of cord dorsum potentials in calves

Adapted from:

Van Soens I., De Vlaminck C., Pille F., Vlaminck L., Duchateau L., Van Ham L., 2015. Recording of Cord Dorsum Potentials in Calves. Research in Veterinary Science, submitted.

Summary

Cord dorsum potentials (CDPs) are sensory evoked potentials that are being used to assess the proximal sensory nerve, the dorsal nerve root and the spinal cord dorsal horn function. The CDP is a spinal cord field potential that arises in the region of the spinal cord segments receiving input from peripheral nerves. The purpose of the present pilot study was to establish normal values for CDP onset latency (OL), peak latency (PL) and peak-to-peak amplitude (PPA) after saphenous nerve stimulation in calves.

CDPs were recorded under general anaesthesia in 15 clinically and neurologically healthy calves. The saphenous nerve was stimulated with 2 monopolar needle electrodes. CDPs were recorded from the interarcuate space L3-L4, L4-L5, L5-L6 and L6-S1 with a monopolar needle electrode. OL was measured as the shortest distance between the trigger point and the takeoff of the initial phase and PL between the trigger point and the peak of the highest phase. PPA was measured between the two largest peaks of opposite polarity.

CDPs were easily recorded at the different recording sites. CDPs consisted of an initial small polyphasic wave and a large negative peak (actual CDP) followed by a long latency positive phase. The largest responses were recorded at the L5-L6 interarcuate space. Body temperature significantly influenced PPA but not OL and PL. OL prolonged with increasing limb length.

Conclusion: CDPs in response to saphenous nerve stimulation in calves were reproducibly recorded in calves at L3-4, L4-L5, L5-L6 and L6-S1 recording sites, with the largest responses at L5-L6.

Introduction

The cord dorsum potential (CDP) is an evoked spinal cord field potential that arises in the dorsal horn interneurons of the spinal cord segments receiving input from a stimulated peripheral nerve (Coombs et al., 1956). CDPs are purely sensory evoked potentials that are being used to assess proximal sensory nerve, dorsal nerve root and spinal cord dorsal horn function.

In veterinary medicine, the technique of recording CDPs and its clinical application has been described in various species, including dogs (Holliday et al., 1979; Cuddon et al., 1999; Cuddon, 2002), cats (Coombs et al., 1956; Cuddon, 2002; Mizisin et al., 2002), rats (Shanker Sharma et al., 1991; Winkler et al., 1998) and horses (van Loon et al., 2010).

In humans and dogs, the CDP consists of three phases: an initial triphasic wave, a large negative wave (the actual CDP) and a long latency positive wave (Cuddon et al., 1999; Kimura, 2001). The initial triphasic wave represents the propagation of sensory action potentials into the spinal cord after stimulating a sensory peripheral nerve. The large negative wave is produced by dorsal horn interneuron activity. The final long latency positive wave represents the primary afferent depolarization.

The purpose of the study was to develop a clinically applicable technique for recording CDPs following stimulation of the saphenous nerve in 15 anaesthetized calves. We hypothesized that CDPs could be consistently measured following stimulation of the saphenous nerve and that normal values for CDP onset latency (OL), peak latency (PL) and peak-to-peak amplitude (PPA) at different intervertebral locations could be established. It was anticipated that these CDP values might potentially be used for assessment of proximal nerve, nerve root and spinal cord dorsal horn integrity.

Materials and methods

CDPs were recorded under general anaesthesia in 15 clinically and neurologically healthy Holstein Friesian calves of 6 to 10 weeks of age. Bodyweight, height at the withers, limb length from trochanter major to calcaneus and body length from T2 to S1 were measured. During measurements, body temperature was maintained in the range between 36°C and 39°C.

Anaesthesia was induced with ketamine (1.8 mg/kg IV, Anesketin, Eurovet) after administration of xylazine (0.2 mg/kg IM, Xyl-M 2%, VMD). Each calf was intubated and general anaesthesia was maintained with isoflurane in oxygen. The animal was placed in lateral recumbency.

A commercially available electrophysiology unit (Sapphire, Acertys Healthcare) was used for peripheral nerve stimulation and recording of CDPs. In each calf, both the right and left saphenous nerves were stimulated with the stimulated limb lowermost. The saphenous nerve was stimulated with 2 monopolar (cathode + anode) needle electrodes (Teflon-coated except for the tip, Acertys Healthcare) placed at the caudal border of the tibia at 2/3 of its length, in close proximity and cranial to the medial saphenous vein. CDPs were recorded at the interarcuate spaces L3-L4, L4-L5, L5-L6 and L6-S1 with a monopolar needle electrode (Acertys Healthcare). A reference electrode (subdermal needle electrode, Acertys Healthcare) was placed 3-4 cm laterally of the detection electrode, contralateral to the stimulating site. The ground electrode was a subdermal needle electrode (Acertys Healthcare) placed over the patella. Sensitivity was set at 5 mV per division. Analysis time was 100 ms following the stimulus. Stimulation intensity was increased until supramaximal stimulation was achieved (maximum of 150 mV). Signal averaging was used until 150 sweeps were recorded. Peak to peak amplitude (PPA) (in mV) was measured between the two largest peaks of opposite polarity. Onset latency (OL) (in ms) was measured as the shortest distance between the trigger point and the takeoff from baseline of the initial phase and peak latency (PL) (in ms) between the trigger point and the peak of the highest phase.

Statistical analyses were performed with statistical software (SAS, version 9.3, SAS Institute Inc, Cary, NC). Three different aspects of the CDP were considered as response variables: PPA, OL and PL. According to the Kolmogorov test, the response variables were normally distributed. Statistical analysis was based on the mixed model inserting calf and limb nested in calf as random effects, and different variables of interest: the categorical fixed effects factor location (L3-L4, L4-L5, L5-L6 and L6-S1), and the continuous fixed effects factors age, bodyweight, height at the withers, limb length, body length and temperature. F-tests were used at a 5% global significance level. Pairwise comparisons between the 4 different locations are adjusted by Tukey's multiple comparisons technique and adjusted P-values are reported.

Results

Each calf was considered clinically normal on the basis of physical and neurological examination findings. The age of the calves varied from 8 ± 2 (mean \pm SD) weeks, bodyweight from 65.6 ± 9.4 kg, height at the withers from 84.9 ± 3.4 cm, limb length from trochanter major to calcaneus from 43.4 ± 2.7 cm, and body length from T2 to S1 from 50.9 ± 4.1 cm.

In each calf CDPs were easily recorded at all recording sites. CDPs consisted of a large negative peak (actual CDP) followed by a long latency positive phase (Figure 13); in some calves polarity was reversed. An initial small polyphasic wave was occasionally observed.

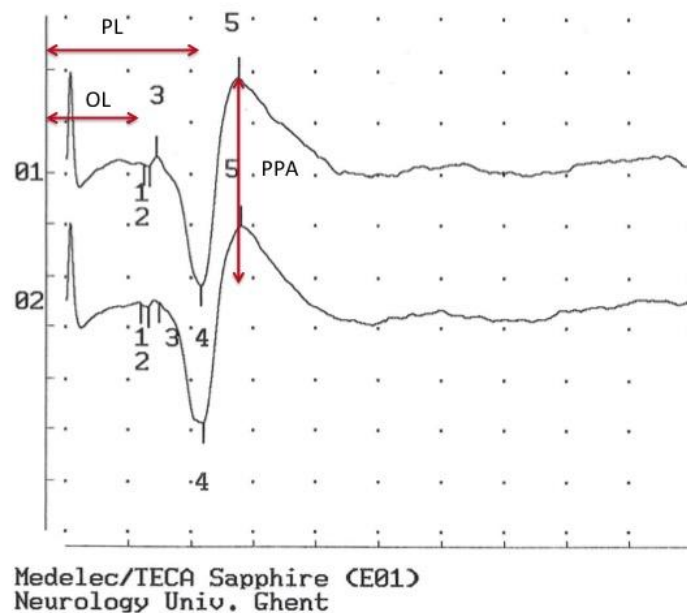


Figure 13 - Cord dorsum potential detected at the L5-L6 interarcuate space after peripheral saphenous nerve stimulation in a calf. OL= onset latency, PL= peak latency, PPA= peak-to-peak amplitude.

Mean (\pm SD) PPA, OL and PL are given in Table 8. Statistically significant differences in PPA were observed between recording sites L3-L4 and L4-L5, L3-L4 and L5-L6, L4-L5 and L6-S1, and L5-L6 and L6-S1. Significant differences in OL were observed between all recording sites except between L4-L5 and L5-L6. For PL, significant differences were measured at all recording sites except between L5-6 and L6-S1.

Age, bodyweight, height and body length did not significantly influence OL, PL and PPA (Table 9). However, body temperature significantly influenced PPA ($p=0.03$); a decrease of 1°C was associated to a decrease of 1,3 mV. Onset latency significantly increased with increasing limb length (for 1 cm increase in length, an increase of 0.18ms was measured) but no influence of limb length on PL or PPA was observed.

Table 8 - Mean (\pm SD) peak-to-peak amplitude (PPA), onset latency (\pm SD) (OL) and peak latency (\pm SD) (PL) of cord dorsum potentials in calves measured at the interarcuate spaces L3-L4, L4-L5, L5-L6 and L6-S1.

Recording site	PPA (in mV)	OL (in ms)	PL (in ms)
L3-L4	3,36 ^a (0.52)	8.01 ^a (0.24)	11.61 ^a (0.17)
L4-L5	7.05 ^b (0.52)	7.59 ^b (0.24)	11.15 ^b (0.17)
L5-L6	8.09 ^b (0.52)	7.39 ^b (0.24)	10.45 ^c (0.17)
L6-S1	3.23 ^a (0.52)	7.65 ^{ab} (0.24)	10.40 ^c (0.17)

Recording sites sharing the same letter in a column do not differ significantly.

Table 9 - The effect of continuous covariates on PPA, OL and PL, represented as slope (standard error), and the P-value for the hypothesis that the slope equals 0.

Covariate	PPA	OL	PL
Age	-0.024 (0.026); p = 0.37	0.009 (0.014); p = 0.54	0.010 (0.009); p = 0.26
Body weight	-0.047 (0.042); p = 0.28	0.015 (0.023); p = 0.53	0.025 (0.014); p = 0.10
Height at the withers	-0.118 (0.119); p = 0.34	0.071 (0.062); p = 0.27	0.075 (0.038); p = 0.07
Limb length	-0.018 (0.145); p = 0.90	0.172 (0.062); p = 0.01*	0.064 (0.048); p = 0.20
Body length	0.062 (0.099); p = 0.54	0.081 (0.049); p = 0.12	0.047 (0.033); p = 0.19
Temperature	1.302 (0.511); p = 0.03*	-0.122 (0.188); p = 0.53	-0.088 (0.215); p = 0.69

* Indicates a significant influence on the continuous covariates PPA, OL, PL (significance level $P < 0,05$)

Discussion

The present study demonstrated that CDP recording after saphenous nerve stimulation is a feasible and clinically applicable technique in healthy anaesthetized calves. The presence of a cord dorsum potential indicates that conducted sensory nerve action potentials evoked from the saphenous nerve have reached the spinal cord lumbar intumescence via the proximal part of the sensory nerve and the dorsal nerve root(s).

CDPs were easily and reproducibly recorded at the L3-L4, L4-L5, L5-L6 and L6-S1 interarcuate spaces in all animals. CDPs consisted of a large negative peak (= actual CDP) followed by a long latency positive phase. This waveform corresponded well to the waveform reported in human and veterinary medicine (Cuddon et al, 1999; Kimura, 2001). The initial small polyphasic wave, as described in humans and dogs, was only occasionally observed. This part of the CDP waveform represents the spread of sensory action potentials into the spinal cord and is of low amplitude. Probably, in most cases, the actual CDP was superimposed on this initial phase.

The largest potentials, i.e. the CDPs with the largest amplitudes were recorded at the L5-L6 interarcuate space. In veterinary literature, the femoral and its saphenous nerve branch is described to originate from L4-L5, L5-L6 and L6-S1 nerve roots in calves (McLeod et al., 1958; Getty, 1975; Nickel et al., 1984; Budras and Habel, 2003; Barone, 2010). As the CDP is generated in the dorsal grey matter, with the largest waveform generated in the spinal cord segments where the stimulated dorsal nerve roots penetrate the spinal cord, we can conclude that the main afferents of the stimulated saphenous nerve enter the spinal cord at the "L5-L6 intervertebral space" in our calves. The presence of CDPs at adjacent intervertebral sites in the different calves is consistent with findings in dogs in a previous study (Holliday et al., 1979). Moreover, the detection of smaller CDPs cranial or caudal to the site of insertion in the spinal cord of the stimulated peripheral nerve might reflect the ability to record CDPs a few centimeters adjacent to the site being investigated (Lindblom and Ottosson, 1953).

In this study, PPAs were extremely variable among calves, making it improbable that PPA could be used as an objective clinical assessment tool of CDPs. PPA of an evoked potential depends on the number of axons recruited. Therefore, stimulation intensity and accessibility of the stimulated nerve are important influencing factors (Chokroverty, 1989; Dull et al., 1990; Jalinous, 1991). Some variation in placement of the stimulating electrode relative to the saphenous nerve might have changed the stimulus intensity of the nerve. Moreover, changes in detection needle placement relative to the spinal cord might play a role in the PPA variability. The larger the distance between the needle and the spinal cord, the smaller the PPA (Campbell et al, 2013).

The overall variability of OL and PL was minimal, indicating the reproducibility of this parameter in calves. Latency times are dependent of different factors such as length of the neural pathway, type of axons being stimulated and conduction velocity and temperature of the limb (Chokroverty, 1989; Dull et al., 1990; Jalinous, 1991, Cuddon, 2002). In the present study limb length had a significant influence on OL; increasing limb length resulting in an increase of OL. This effect was not found on PL; as the configuration of the CDP considerably varied at the different recording sites, it might have been less accurate to define the exact site of the PL. Therefore we conclude that OL is a more accurate parameter to interpret CDPs.

The influence of body temperature on different parameters of nerve conduction studies is well known in literature (Morris, 2013). A significant influence of body temperature on PPA was observed in the calves of this study; a decrease in temperature resulted in a decrease in PPA. No significant effect on latency times was observed in the calves of the present study, indicating again the value of this parameter in CDP studies in calves.

Conclusion

CDPs were recorded from all calves tested in the study; responses with the largest amplitudes after saphenous nerve stimulation were detected at the L5-L6 interarcuate space. Onset latency seems the most valuable parameter to interpret CDP recordings. This technique may be useful as a tool to assess the functional integrity of the proximal segment of peripheral nerves, the dorsal nerve roots and the dorsal horns of the spinal cord in calves.

References

- Barone R., 2010. Anatomie comparée des mammifères domestiques, Vol.7 : Neurologie II : Vol.7 : Neurologie. Editions Vigot, Paris, France, p 161.
- Budras K., Habel R.E., 2011. Bovine Anatomy. 2ed., Schlütersche, Hannover, Germany, p 184.
- Campbell J.O., Olby N.J., Hash J.A., Lascelles B.D., 2013. Assessment of cord dorsum potentials from caudal nerves in anesthetized clinically normal adult dogs without or during neuromuscular blockade. American Journal of Veterinary Research 74, 616-620.
- Chokroverty S., 1989. Magnetic stimulation of the human peripheral nerves. Electromyography and clinical neurophysiology 29, 409-416.
- Coombs J.S., Curtis D.R., Landgren S., 1956. Spinal cord potentials generated by impulses in muscle and cutaneous afferent fibres. Journal of Neurophysiology 19, 452-467.
- Cuddon P.A., Delauche A.J., Hutchison J.M., 1999. Assessment of dorsal nerve root and spinal cord dorsal horn function in clinically normal dogs by determination of cord dorsum potentials. American Journal of Veterinary Research 60, 222-226.
- Cuddon P., 2002. Electrophysiology in neuromuscular disease. Veterinary Clinics of North America: Small Animal Practice 32, 31-62.
- Dull S., Konrad P., Tacker W., 1990. Peak-to-peak amplitude and latency characteristics of spinal cord motor evoked potentials in the rat. Electroencephalography and clinical neurophysiology 77, 68-76.
- Getty R., 1975. Sisson and Grossman's The Anatomy of the Domestic Animals. 5ed., W.B. Saunders Co, Philadelphia, Pennsylvania, USA, p 1141.
- Holliday T.A., Weldon N.E., Ealand B.G., 1979. Percutaneous recording of evoked spinal cord potentials of dogs. American Journal of Veterinary Research 40, 326-333.

- Jalinous R., 1991. Technical and practical aspects of magnetic nerve stimulation. *Journal of Clinical Neurophysiology* 8, 10-25.
- Kimura J., 2001. *Electrodiagnosis in Diseases of Nerve and Muscle: Principles and variations of nerve conduction studies*. Oxford University Press, USA, pp 91-129.
- Lindblom U.F., Ottosson J.O., 1956. Bulbar influence on spinal cord dorsum potentials and ventral root reflexes. *Acta Physiologica Scandinavia* 20, 203-214.
- McLeod, W.M., Trotter, D.M., Lumb J.W., 1958. *Bovine Anatomy*. 2ed., Burgess Publishing Company, Minneapolis, Minnesota, USA, p 232.
- Mizisin A.P., Shelton G.D., Burgers M.L., Powell H.C., Cuddon P.A., 2002. Neurological complications associated with spontaneously occurring feline diabetes mellitus. *Journal of Neuropathological Experimental Neurology* 61, 872-884.
- Morris J., 2013. Technical tips: methods of warming and maintaining limb temperature during nerve conduction studies. *Neurodiagnostics Journal* 53, 241-251.
- Nickel R., Schummer A., Seiferle E., 1984. *Lehrbuch der Anatomie der Haustiere*, Band 1, Bewegungsapparat. 5ed., Paul Parey, Berlin, Germany, p 425.
- Shanker Sharma H., Winkler T., Stalberg E, Olsson Y., Dey P.K., 1991. Evaluation of traumatic cord edema using evoked potentials recorded from the spinal epidural space. *Journal of Neurological Sciences* 102, 150-162.
- Van Loon J.P.A.M., Van Oostrom J., Doornenbal A., Hellebrekers L.J., 2010. Lumbosacral spinal cord somatosensory evoked potentials for quantification of nociception in horses. *Equine Veterinary Journal* 42, 255-260.
- Winkler T., Sharma H.S., Stalberg E., Badgaiyan R.D., Alm P., Westman J., 1998. Spinal cord evoked potentials and edema in the pathophysiology of rat spinal cord injury. *Amino Acids* 14, 131-139.

SECTION 5.2

Selective dorsal rhizotomy to tackle quadriceps femoris muscle spasticity in cattle: a pilot study

Adapted from:

De Vlaminck C., Van Soens I., Tshamala M., Simoens P., Viaene A., Van Ham L., Pille F., Vlaminck, L., 2015. Selective dorsal rhizotomy as an experimental treatment for spasticity of the quadriceps femoris muscle in cattle. *Veterinary Surgery*, submitted.

Summary

The technique of selective dorsal rhizotomy was evaluated as a treatment option for calves with quadriceps femoris muscle spasticity. Two sound calves and two calves suffering from bilateral hind limb spastic paresis with quadriceps femoris muscle involvement were used for the present prospective study. All calves underwent a laminectomy and unilateral selective dorsal rhizotomy for deafferentation of the quadriceps femoris muscle. Pre- and postoperatively, the saphenous nerve was electrically stimulated and cord dorsum potentials were recorded. Rehabilitation of the calves was evaluated during one month. Autopsy was performed to verify the spinal nerve rootlets that were surgically destroyed and to locate the position of spinal cord segments of interest relative to the vertebral segments. Following selective dorsal rhizotomy, cord dorsum potentials were absent in all operated limbs, except in one sound calf. After a short rehabilitation, both sound calves regained normal locomotor function. The gait of the first spastic calf improved compared to the gait before surgery. However, in the second spastic calf dorsal rhizotomy resulted in severe incoordination and weakness of the hind quarters. Autopsy revealed considerable variation in the location of the L3-L6 neural spinal cord segments in relation to the respective lumbar vertebrae.

Conclusions: In sound calves the procedure of selective dorsal rhizotomy does not cause any permanent locomotor problem. In spastic calves, selective dorsal rhizotomy should be regarded as an experimental procedure for treatment. With a more accurate identification of the neural segments, the outcome of the procedure may be optimized.

Introduction

Treatment of spastic paresis in beef cattle that are not kept for breeding purposes is justified as the physical condition of affected animals deteriorates rapidly due to the constant pain and stress evoked by the muscle spasms. In cattle with spastic paresis of the gastrocnemius muscle, selective tibial neurectomy or selective gastrocnemius tenectomy are the treatments of choice (Bouckaert and De Moor, 1966; Pavaux et al., 1985; Vlaminck et al., 2000). Both techniques are moderately invasive and straightforward. However, to date no adequate treatment is described for spastic paresis of muscles other than the gastrocnemius. In animals suffering from bovine spastic paresis of the quadriceps femoris muscle (BSP-Q) or with multiple muscle involvement (BSP-M), neurectomy or chemical destruction of the femoral nerve is not feasible as it may induce total paralysis of the quadriceps femoris muscle, making the animal unable to bear weight on the limb (De Vlaminck et al., 2013).

As mentioned in the introduction of this thesis, it is believed that an overactive stretch reflex is responsible for the symptoms of bovine spastic paresis (De Ley and De Moor, 1977; De Ley and De Moor, 1980). This hypothesis was shown in three calves by disappearance of symptoms after selective resection of dorsal spinal roots of the tibial nerve at the level of the L4, L5 and L6 vertebrae (De Ley and De Moor, 1977). Hence, the motor fibers in the ventral nerve roots remained intact while the sensory fibers of the dorsal roots were resected in order to eliminate the stretch reflex without denervation of the muscle. In another study, spastic paresis symptoms could be masked after selective suppression of gamma motor neurons within the tibial nerve by epidural administering of a 0.38 per cent procaine solution (De Ley and De Moor, 1980). This concentration of procaine only blocks the thinner gamma motor neurons while leaving nerve fibers of larger diameter (alpha motor and Ia sensory neurons) functionally intact. Based on these studies, it was concluded that spastic contractions of the

gastrocnemius muscles might be caused by overstimulation and/or lack of inhibition of gamma motor neurons.

By performing a selective dorsal rhizotomy, the overactive stretch reflex loop is interrupted. During this procedure, selected sensory fibers (dorsal nerve rootlets) are resected intradurally, but fibers of the ventral roots remain intact in order to eliminate the stretch reflex without denervation of the muscle.

In children with spasticity related to cerebral palsy, selective dorsal rhizotomy is a well-established surgical procedure for improving lower extremity spasticity (Roberts, 2013). The standard technique requires a L1–S1 laminectomy and opening of the dura mater for visualization of the dorsal nerve roots. Following identification by electrical stimulation of the sensory nerve rootlets of interest, rootlet transection results in reduced spasticity without inducing paralysis of the affected muscles.

This experimental study evaluates selective rhizotomy of the dorsal roots of the femoral nerve in two sound calves and in two calves suffering from spastic paresis involving the quadriceps femoris muscle (BSP-Q or BSP-M). It was hypothesised that this technique would not change the locomotor function of the sound animals and would result in complete or partial disappearance of spasticity and improved ambulation in the BSP-Q and BSP-M affected animals, respectively.

Materials and Methods

Animals used in the clinical study

The study design was approved by the Ethical Committee for Animal Research of the Ghent University (EC 2013-154). Two healthy male calves (calf 1 and calf 2) and two calves suffering from bilateral spastic paresis (calf 3, female and calf 4, male) were used.

Gait and/or locomotor function of the different animals were evaluated when rising from sternal recumbency, at rest while standing and when walking in a straight line on a hard surface. In the spastic calves, involvement of the quadriceps femoris muscle was confirmed by performing unilateral anaesthesia of the femoral nerve using a dorsal paravertebral approach (De Vlamynck et al., 2013).

Recording of cord dorsum potentials

Cord dorsum potentials (CDPs) are purely sensory evoked potentials that can be measured to assess integrity and normal function of the proximal segment of a sensory nerve and the corresponding dorsal nerve root and dorsal horn segment of the spinal cord (Cuddon et al., 1999). This technique is routinely used in dogs and the current procedure has been standardized in calves as mentioned in Chapter 5.1.

In the present study, cord dorsum potentials of the femoral nerve were recorded pre-operatively and immediately following closure of the wound after selective dorsal rhizotomy in all 4 calves. The procedure was performed under general anaesthesia as described in Chapter 5.1. The saphenous nerve, which is the peripheral sensory branch of the femoral nerve, was stimulated bilaterally with 2 monopolar needle electrodes placed at the caudo-medial border of the distal tibia, in close proximity and cranial to the medial saphenous vein. Potentials

were recorded with a monopolar needle electrode centrally positioned in the L3-L4, L4-L5, L5-L6 and L6-S1 interarcuate spaces, respectively. During the pre-operative recordings, the distances from the L6-S1 intervertebral space to the location of the needle in the L3-L4, L4-L5 and L5-L6 interarcuate spaces were measured to be able to identify these interarcuate spaces during the CDP recordings following the surgical procedure. Sensitivity was set at 5 mV per division. Analysis time was 100 ms following the stimulus. Stimulation intensity was increased until supramaximal stimulation was achieved (maximum of 150 mV). Signal averaging was used until 150 sweeps were recorded. Each recording was repeated once to assess reproducibility.

Surgical procedure: unilateral selective dorsal rhizotomy

After sedation with xylazine (0.2 mg/kg IM), general anaesthesia was induced with ketamine (1.8 mg/kg IV) and maintained with isoflurane in oxygen. All calves were medicated with cefquinome (1 mg/kg IV), meloxicam (0.5 mg/kg IV) and dexamethasone (0.05 mg/kg IV).

All surgeries were performed by an experienced neurosurgeon (M. Tshamala). The calves were positioned in left lateral recumbency and the level of laminectomy was determined after correct identification of the L2 – L6 spinous processes by palpation and/or ultrasound. The laminectomy was performed as described in literature (Hurov, 1985; Funkquist, 1970; Wheeler, 1994). Following a dorsal midline incision over the spinous processes, the epiaxial musculature was subperiosteally elevated and was retracted laterally. The spinous processes were removed with a rongeur, along with the interconnecting supraspinous and interspinous ligaments. The lamina was removed bilaterally with a high-speed bur, leaving the inner cortical bone intact. Using a Kerrison rongeur, the remaining bone layer was carefully removed and the edges of the fenestrated vertebrae were smoothed. The epidural fat was exposed and wiped away to reveal the surface of the dura. After bleeding from the epidural veins and bone was controlled, the dura was incised to expose the right dorsal rootlets. The dorsal rootlets of the exposed neural segments were identified and isolated with

suture loops. To identify reciprocity with the right quadriceps femoris muscle, the different rootlets were electrically stimulated one by one with a double pole surgical nerve stimulator while the quadriceps femoris muscle was closely observed, palpated and evaluated electromyographically with a concentric needle for contractions. The rootlets for which stimulation resulted in proper contractions of the quadriceps femoris muscle were coagulated with a bipolar electrocauter and subsequently cut. The dura mater was closed in a continuous pattern with a nonabsorbable polyester suture (Ethibond Excel, USP 5/0, Ethicon). Wound closure was performed with a simple continuous suture, using an absorbable polydioxanone suture (PDS II, USP1, Ethicon) for the fascia, and an absorbable polyglactin 910 suture (Vicryl, USP 0, Ethicon) for the subcutaneous tissues. The skin was closed using an absorbable polyglyconate suture (Maxon, USP 0, Covidien).

After completion of post-operative CDP recordings (see above), a stent bandage was applied.

Postoperative care and follow-up

The calves were housed in individual boxes bedded with a mixture of wood shavings and straw. Post-operatively, all calves were further medicated with cefquinome (1 mg/kg IM bid, for 14 days), meloxicam (0.5 mg/kg IV sid, for 5 days) and dexamethasone (0.05 mg/ kg IM sid, for 3 days). Wound care was routinely performed and the wounds were protected with adhesive stents for 12 days. This period was prolonged in case of wound complications. Rehabilitation started the first day after surgery and was continued for one month. The calves were assisted to walk three times a day until they were able to walk without help. Gait and locomotor function was evaluated daily during the complete rehabilitation period.

Post-mortem examination

One month after surgery the calves were euthanized. Careful dissection of the vertebral column and spinal cord was performed to identify the different neural segments pertaining to the transected rootlets and to define the exact position of these neural segments relative to their respective vertebrae. Samples of the femoral nerves, saphenous nerves, quadriceps femoris muscles, spinal cord and spinal ganglia of the L2-L6 region were taken for histological examination to detect signs of neural degeneration following dorsal rhizotomy. Spinal cord and spinal ganglia were fixated in bouin solution. The other tissues were fixated in 4% formaldehyde solution. After embedding and sectioning, transverse sections were mounted on glass slides, and stained with hematoxylin–eosin for microscopic examination.

Results

Animals used in the clinical study

Patient details including breed, age, gender and weight are shown in Table 10. Calves 1 and 2 were free of gait abnormalities or any other locomotor dysfunction, whereas both calves 3 and 4 had a history of bilateral hind limb spasticity since birth. Calf 3 showed a very small and compact body conformation. From time to time, it needed assistance to get up. When standing, the calf was continuously weight shifting in the hind limbs while stretching these limbs in a cranial direction. When walking, a cranially directed pendulous movement of the affected hind limbs together with an increased tonus of the tail was recorded. Spasticity of the right hind limb disappeared after performance of a right femoral nerve block as described by De Vlamynck et al. (2013), confirming BSP-Q in this calf. Calf 4 was a well-muscled calf. Most of the times it was found in a dog-sitting posture with stretched hind limbs and was not able to get up on its own. It could remain standing for only a few minutes and was constantly weight shifting in the hind limbs while stretching these limbs in a cranial direction and occasionally in a lateral direction. While walking, the calf showed a cranially directed pendulous movement of the hind limbs with an increased tonus of the tail. Spasticity of the right hind limb partially decreased after performance of a right femoral nerve block. However, the calf now needed extra tail support to be able to walk. Spastic contractions of the right hind limb in lateral direction were still present after the femoral nerve block. Therefore the calf was classified as a BSP-M case.

Table 10 - Data of 4 calves (2 healthy, 2 affected) that underwent right-sided selective dorsal rhizotomy. Breed, age, gender, weight and duration of the rehabilitation period are shown. HF = Holstein Friesian, BB = Belgian Blue.

Calf nr	Breed	Gender	Healthy/ Affected	Age at surgery (days)	Weight at surgery (kg)	Rehabilitation Period (days)
Calf 1	HF	Male	Healthy	42	55	34
Calf 2	HF	Male	Healthy	24	54	34
Calf 3	BB	Female	Affected BSP-Q	89	45	29
Calf 4	BB	Male	Affected BSP-M	32	87	30

Recording of cord dorsum potentials

CDPs were recorded pre- and post-surgery as described before. Immediately following surgery, CDPs were absent when stimulating the operated right side in all animals except in calf 2, in which CDPs were recorded in the L3-L4, L4-L5, L5-L6 and L6-S1 interarcuate spaces. Normal CDP readings were recorded in the untreated hind limbs of all animals.

Surgical procedure: unilateral selective dorsal rhizotomy

In calf 1 exposure of femoral nerve L4-L6 neural segments was attempted by laminectomy of L4 to L6 vertebrae, based on anatomical data (McLeod, et al., 1958; Getty, 1975; Seiferle, 1992; Barone, 2010; Budras and Habel, 2011). Electrical stimulation identified femoral nerve dorsal rootlets at the L4-L5 intervertebral level. Dorsal roots stimulated at the level of both the L5 and L6 vertebrae corresponded with more distal muscle groups (Table 11). Based on the experiences with calf 1, in calf 2 laminectomy was performed from L2 to L5. Based on the results of electrical stimulation of the different exposed dorsal rootlets in calf 2, laminectomy of vertebrae L3 to L5 was performed in calves 3 and 4.

The peroperative identification of the different neural segments based on electrical stimulation of exposed dorsal rootlets is summarized in Table 11. All rootlets that showed clear reciprocity with the quadriceps femoris muscle at electrostimulation were cauterised and cut, while in calf 3 dorsal rootlets communicating with the m. psoas major were also accidentally transected.

In all calves, bleeding from the surgically manipulated vertebrae or the epidural veins was difficult to control. Several actions were taken to improve hemostasis: Bone Wax (SMI, Belgium) was applied over the bleeding bone and the surgery table was tilted with the hindquarters elevated to lower blood pressure in this region. Moreover, the mean blood pressure was maintained low by adapting the depth of anaesthesia.

Due to the restricted width of the laminectomy window, visualising the different dorsal rootlets was not straightforward, time-consuming and allowing only access to the most medial parts of the rootlets.

Table 11 - Overview of the dorsal roots of the four calves.

Each dorsal root (L2-S1) is divided in 3 regions (cranial, mid, caudal). Orange: stimulated and transected rootlets; green: stimulated rootlets left intact; blue: not stimulated rootlets. Stimulation of dorsal rootlets resulted in contraction of quadriceps femoris muscles (Q), psoas major muscle (PM), abdominal wall muscles (ABD), digital extensor muscles (EXT), gastrocnemius muscle (G), adductor muscles (ADD) and muscles of the rump (R). The dorsal rootlets of L4, L5 and L6 that theoretically may correspond with the quadriceps femoris muscle are marked with dots. Complete stimulation of all of these rootlets was not performed in any of the four calves.

			Calf ID			
			Calf 1	Calf 2	Calf 3	Calf 4
Level of dorsal spinal roots	L2	Cranial		ABD		
		Mid		ABD		
		Caudal		ABD		
	L3	Cranial		PM		
		Mid		ABD		
		Caudal		ABD	PM	
	L4	Cranial		PM	PM	Q
		Mid		Q	Q	Q
		Caudal			Q	Q
	L5	Cranial	Q	Q		Q
		Mid	Q	Q		EXT
		Caudal	Q	Q		
	L6	Cranial	EXT + R			
		Mid	R			
		Caudal	ADD			
	S1	Cranial	anus + R			
		Mid	G			
		Caudal	G			

Postoperative care and follow-up

Following recovery from anaesthesia, calf 1 was able to rise when supported by the tail. The next day the calf was able to rise on its own, but at walk proprioceptive deficits were noted in the right hind limb. This condition improved by eight days after surgery, but when standing the calf was often observed bearing weight on the dorsal aspect of the fetlock of the right hind limb (Figure 14). These symptoms also gradually disappeared, and by twelve days after surgery the calf was standing and ambulating perfectly normally. Seven days after surgery, the calf gradually developed a subcutaneous surgical site infection that resulted in abscess formation. Drainage of the abscess at 14 days after surgery was followed by daily wound care. A methicillin resistant *Staphylococcus aureus* was isolated after culture. The calf ambulatory capacities were not influenced by this complication.

Following recovery from anaesthesia, calf 2 was able to rise without help. When walking, it positioned the hind limbs normally, but the stride length of the right hind limb was shortened. This condition gradually improved and resulted in a normal gait six days postoperatively. The surgical wound healed without complications.

Calf 3 was also able to rise without external assistance following recovery from anaesthesia. When walking, the animal showed no pendulous movements anymore in the right hind limb, although the limb was advanced without hip flexion. Signs of spasticity in the untreated limb were unchanged in comparison to the pre-surgical condition. No further improvement of ambulation was observed over the rehabilitation period. The surgical site healed without complications.

During the rehabilitation period, calf 4 was only able to get up with tail assistance. Supported by the tail, it was able to stand and walk for a short distance. The animal was still frequently observed in sternal or dog-sitting posture with the hind limbs stretched cranially as before surgery. When standing, the calf had no control over the position of both hind limbs. While walking, clear weakness of the hindquarters was seen the first days after surgery. This evolved further to increased muscle tone in both hind limbs resulting in reappearance of

the preoperative pendulous movement. The surgical wound healed without complications.

Figure 14 - Proprioceptive deficit of the right hind limb in calf 1 when walking shortly after surgery. A bandage was placed on the fetlock of the right hind limb to prevent from pressure wounds.



Post-mortem examination

Adhesions between dura and pia mater were observed in the surgical area in all calves. In calf 1, the surgical site infection was still present at the time of autopsy and involved the subcutaneous and superficial muscular layers throughout the complete length of the incision. Additionally, adhesions were found between the dura mater and the epiaxial muscles as well as extradural oedema over the right side of the spinal cord. Diffuse extradural and intradural oedema was observed in calf 3. In calf 4, a small incisional intermuscular abscess was located at the level of the L5 vertebra. Bacteriological examination was not performed.

Considerable individual variation was observed in the location of the L3-L6 neural segments relative to the L3-L6 vertebral bodies. This resulted in incomplete exposure of all appropriate L4-L6 neural segments in all calves. Topography of the neural segments of interest relative to surrounding bony structures of calf 4 is shown in Figure 15. During autopsy, the denticulate ligament was used to identify the borders between neural segments L2-L3, L3-L4, L4-L5 in each calf. Between L5 and L6 and between the more caudal neural segments, this ligament was not observed and the differentiation between the subsequent roots was based on the penetration of the roots through the dura mater. In the L2-L3, L3-L4 and L4-L5 neural segments convergence of the separate dorsal rootlets was observed, whereas starting from neural segment L6, the dorsal rootlets of each root formed a compact bundle with a progressively longer caudal intradural course (Figure 16).

No neural or muscular cell degeneration was recorded in any of the histologically examined tissue samples.

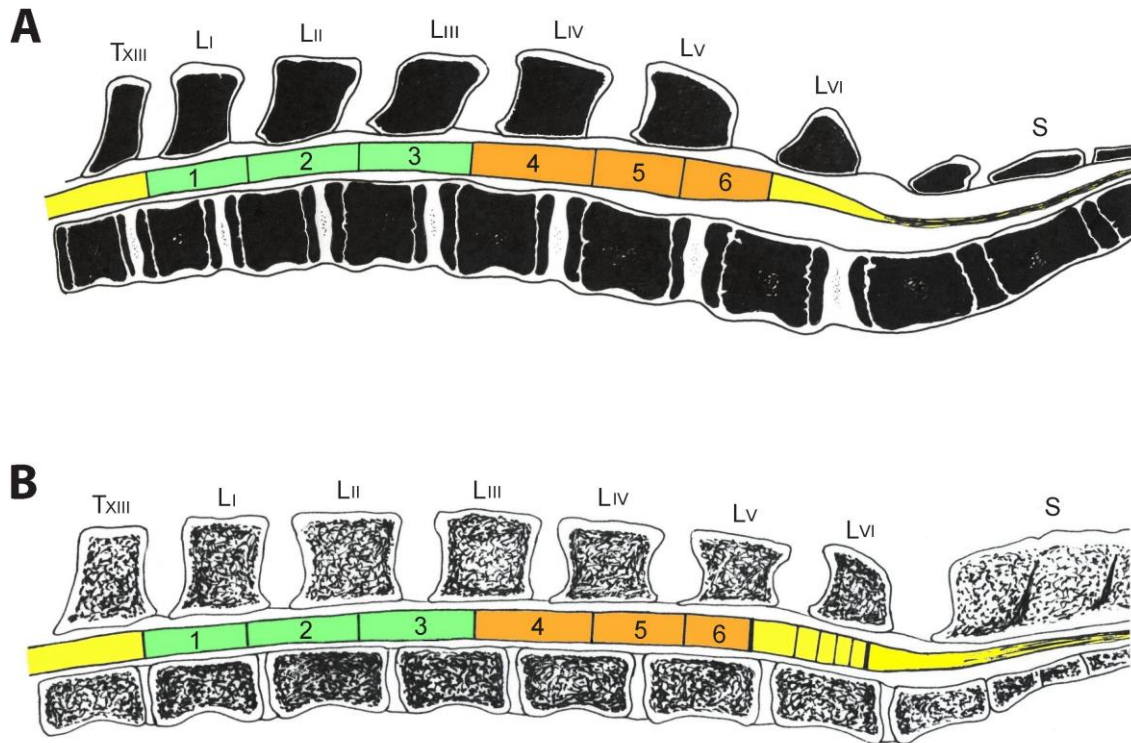


Figure 15 - Median section through the spinal column showing the topography of the lumbar spinal cord segments.

A. Calf 4, 32 days old

B. Adult cattle (according to Seiferle, 1992)

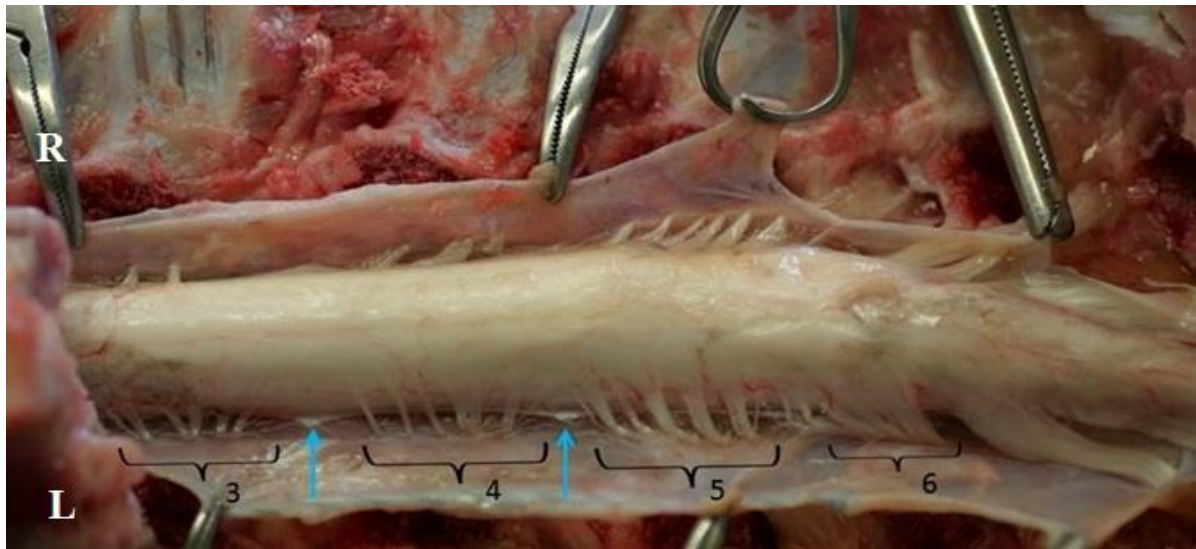


Figure 16 - Dorsal intradural view of the spinal cord of calf 3. On the right side, the caudal dorsal nerve rootlets of the L3 neural segment and all dorsal nerve rootlets of the L4 neural segment were surgically destroyed. The right ventral nerve rootlets of the L4 neural segment are visible. R: right, L: left; Blue arrows: denticulate ligaments; Braces with nr. 3, 4, 5 and 6 mark the L3, L4, L5 and L6 neural segments, respectively. The denticulate ligament was used to identify borders between the L2-L3, L3-L4 and L4-L5 neural segments in each calf. This ligament was not visible between L5-L6 nor between the more caudal neural segments. Notice the convergence of the separate dorsal rootlets in the L3-L5 neural segments; in contrast, starting from the L6 neural segment, the dorsal rootlets of each root form a compact bundle with a progressively longer caudal intradural course.

Discussion

Peroperative electrical stimulation of individual rootlets combined with palpation and recording of electromyographic activity of quadriceps femoris muscle parts proved to be successful in identifying femoral nerve dorsal rootlets in this study. As expected, unilateral selective dorsal rhizotomy of the femoral nerve in both healthy calves (nr.1 and nr.2) resulted in temporary proprioceptive deficits that spontaneously and completely resolved over a limited time period. This can be attributed to the interruption of the proprioceptive neural path by sectioning the dorsal roots. However, this was only a temporary effect with complete normalization following a short rehabilitation period. The same unilateral technique performed on a bilaterally BSP-Q affected animal (nr.3) resulted in almost complete resolution of clinical symptoms in the operated limb. The BSP-M affected animal (nr.4), however, showed deterioration of its ambulation following this surgical intervention. The lack of a control group with sound calves in which a laminectomy was performed but not a rhizotomy, was the main limitation of this study.

The inability of the BSP-Q calf to flex the hip of the operated limb is suggestive of a defective motor function of the m. tensor fasciae latae and/or the m. psoas major (Budras and Habel, 2011). A defective motor function of the m. psoas major is most likely to have caused the symptoms as rootlets corresponding with this muscle were accidentally sectioned during surgery. However, these clinical signs are difficult to explain because loss of dorsal root continuity should not alter the muscular motor function.

In the BSP-M calf, surgery was not successful. Several hypotheses may explain this result. Firstly, lack of residual voluntary muscle strength of the quadriceps femoris muscle might have caused the gait deterioration, coordination disturbances and weakness of the hind quarters after surgery, as deterioration of ambulation following selective dorsal rhizotomy has also been seen in children

with cerebral palsy because of a lack of residual voluntary muscle strength of anti-gravitational limb muscles (Vaughan et al., 1998; Van Schie et al., 2005; Steinbok, 2006; Gump et al., 2013; Grunt et al., 2014). For these patients, preoperative tests have been developed to evaluate the strength of different muscles, gross motor function and gait pattern before deciding whether the surgery be performed or not (Vaughan et al., 1998; Van Schie et al., 2005; Steinbok, 2006; Gump et al., 2013; Grunt et al., 2014). Unfortunately, to date, in animals no objective tests exist to assess the function of the different muscle groups that can be involved in spastic paresis. Secondly, the calf might have suffered from a supplementary disorder such as dystonia, resulting in increased muscle tone in which the stretch reflex loop is not affected. Dystonia is a neurological movement disorder that causes involuntary muscle contractions, resulting in twisting, repetitive and patterned movements as well as abnormal postures (Steinbok, 2006). The pathogenesis of dystonia is unknown, but a disorder in the basal nuclei of the brain is suspected. While the stretch reflex loop is modulated during selective dorsal rhizotomy, the procedure does not alleviate symptoms in children suffering from dystonia (Gump et al., 2013; Roberts, 2013).

Despite incomplete deafferentation of the right femoral nerve in both spastic calves, good response to treatment was recorded in the BSP-Q animal, contrary to what was observed in the BSP-M calf. Based on a clinical examination it is impossible to determine which muscle groups can be involved in animals with BSP-M. The femoral nerve block can be helpful in the differentiation between BSP-Q and other BSP-animals, as mentioned in Chapter 4.2. Selective rhizotomy might be an additional tool in an experimental setting to study the pathogenesis of complex spasticity in cattle as performed by De Ley and De Moor (1977) when investigating spasticity of the gastrocnemius muscle.

In all calves of the present study, deafferentation of the femoral nerve was incomplete, most likely as a result of the variable location of the neural segments and the limited exposition of the different rootlets. The femoral nerve originates from the fifth lumbar nerve, with smaller contributions from the fourth and sixth lumbar nerve. According to Seiferle (1992), in adult cattle the L4, L5 and L6

neural segments are located at the L3-L4 intervertebral level, the L4-L5 intervertebral level and the L5 vertebral body level, respectively. The autopsy results of the present study illustrate discrepancies with these data. In fact, based on the limited topographic results from the present study, it might be concluded that laminectomy of L3, L4 and L5 should be combined with additional partial laminectomy of the cranial part of the L6 vertebra to expose all dorsal rootlets of the L4-L6 neural segments. Two hypotheses for the discrepancies are postulated. Firstly, it is known that as a result of differential growth of the spinal cord and the vertebral column, the spinal cord ascends with growth and in our study young calves were used in which the ascent of the spinal cord was still progressing (Budras and Habel, 2011). Therefore, the well-documented topography of the spinal cord in adult cattle is not representative for the calves in our study, while scientific reports of the topography of neural segments in growing cattle are lacking (Figure 17). Secondly, in literature topography of neural segments in adult cattle is described relative to the vertebral bodies (Seiferle, 1992; Barone, 2010). However, during laminectomy the spinous processes are used as landmarks for the neural segments, but these processes are not located precisely on top of the corresponding vertebral bodies, making the topographic description of neural segments in literature ineffective as a base for the approach to the neural segments of interest during surgery. Therefore, topographic research of the lumbar neural segments relative to the spinous processes and vertebral arches in calves of different ages and different breeds are necessary to be able to establish a correct approach to the spinal cord regions of interest in young animals.

In the present study, visualization of the surgically exposed rootlets was limited due to persistent diffuse hemorrhage and the limited size of the window that can be safely created during laminectomy to prevent postoperative spinal column instability and spinal cord compression. As a result, the rhizotomy procedure is very time-consuming with surgery times easily reaching 4 hours. In man, selective dorsal rhizotomy for treatment of spasticity may also be performed by endoscopic assistance, resulting in minimal invasive surgery (Fonoff et al., 2011). Hence, the endoscopic procedure may result in a more accurate identification of the femoral nerve dorsal rootlets and a shorter surgery time.

However, besides the endoscope, special instruments are required for this procedure, such as isolated electrodes for electrical stimulation testing and equipment for radiofrequency selective ablation of nerve rootlets (Fonoff et al., 2011).

Wound infection, encountered in 2 out of 4 animals in this study, was considered an important complication, despite its localization in the superficial soft tissues. This is attributed to the prolonged surgery time and subsequent exposure of soft tissues, which is known to influence surgical site infection rates (Fuller, 2013). Appropriate measures should be taken to reduce the infection incidence.

Cord dorsum potential recordings are advantageous in animals suspected of sensory neuropathy, dorsal radiculopathy, nerve root injury or dorsal horn myelopathy involving the cervical or lumbar intumescence (Cuddon et al., 1999). In this study cord dorsum potentials were measured using a technique adapted from canine medicine to objectively evaluate proper deafferentation of the femoral nerve (Cuddon et al., 1999). Despite incomplete deafferentation in calves 1, 3 and 4, no postoperative CDP were recorded. This may be due to the fact that stimulating the saphenous nerve does not result in electrical excitement of all associated femoral nerve rootlets (Ranck, 1975). In calf 2, probably some of the femoral nerve rootlets were left intact (as neural segment L6 was not exposed) which might account for the positive CDP reading in this animal following surgery.

Histological examination of different neural tissue samples did not show degenerative changes following rhizotomy. This is in accordance with Bloom & Fawcett (1975) who also found that sectioning of dorsal nerve roots does not result in detectable histological changes of sensory ganglion cells.

Conclusion

As shown in sound animals, selective dorsal rhizotomy can be performed without permanent locomotor deficits. At this stage, selective dorsal rhizotomy seems to be the only possible surgical treatment for bovine spastic paresis involving the quadriceps femoris muscle. Based on the observations of the present study, performing a laminectomy of the L3-L5 vertebrae and the cranial half of the L6 vertebra should expose the L4 to L6 neural segments that may have reciprocity with the quadriceps femoris muscle. Because of the considerable individual and age-dependent variation in the location of the neural segments relative to the vertebrae and because of the difficulty to discriminate subsequent roots, the use of peroperative electrostimulation is essential to identify those rootlets that have reciprocity with the quadriceps femoris muscle. It is tempting to speculate that with a more accurate identification of the rootlets, the outcome of the procedure can be optimized. Although the surgical procedure as well as the rehabilitation is currently too demanding to advocate rhizotomy for clinical use, experimental muscle deafferentation seems an interesting technique for unravelling the pathogenesis of complex spasticity in cattle.

References

- Barone R., 2010. Anatomie comparée des mammifères domestiques, Vol.7 : Neurologie II : Vol.7 : Neurologie. Editions Vigot, Paris, France, p 161.
- Bloom W., Fawcett D.W., 1975. A textbook of histology. 10ed., Saunders, Philadelphia, Pennsylvania, USA, p 1033.
- Bouckaert J.H., De Moor A., 1966. Treatment of spastic paralysis in cattle: improved denervation technique of the gastrocnemius muscle and post-operative course. *Veterinary Record* 79, 226-229.
- Budras K, Habel R.E., 2011. Bovine Anatomy. 2ed., Schlütersche, Hannover, Germany, p 184 .
- Cuddon P.A., Delauche A.J., Hutchison J.M., 1999. Assessment of dorsal nerve root and spinal cord dorsal horn function in clinically normal dogs by determination of cord dorsum potentials. *American Journal of Veterinary Research* 60, 222-226.
- De Ley G., De Moor A., 1977. Bovine spastic paresis: results of surgical desafferentation of the gastrocnemius muscle by means of spinal dorsal root resection. *American Journal of Veterinary Research* 38, 1899-1900.
- De Ley G., De Moor A., 1980. Bovine spastic paralysis: results of selective γ -efferent suppression with dilute procaine. *Veterinary Science Communications* 3, 289-298.
- De Vlamynck C., Vlaminck L., Hauspie S., Saunders J., Gasthuys F., 2013. Ultrasound-guided femoral nerve block as a diagnostic aid in demonstrating quadriceps involvement in bovine spastic paresis. *The Veterinary Journal* 196, 451-455.
- Fonoff E.T., Contreras Lopez W.O., Almeida de Oliveira Y.S., Alves Lara N., Jacobsen Teixeira M., 2011. Endoscopic Approaches to the Spinal Cord. In: Alexandre, A., Masini, M., Menchetti, P.P.M., *Advances in Minimally Invasive Surgery and Therapy for Spine and Nerves*. 1ed., Springer-Verlag/Wien, Germany, pp 75-84.

- Fuller J.K., 2013. *Surgical Technology: principles and practice*. 6ed., Saunders, St. Louis, Missouri, USA, p 1053.
- Funkquist B., 1970. Decompressive laminectomy in thoracolumbar disc protrusion with paraplegia in the dog. *Journal of Small Animal Practice* 11, 445-451.
- Getty R., 1975. *Sisson and Grossman's The Anatomy of the Domestic Animals*. 5ed., W.B. Saunders Co, Philadelphia, Pennsylvania, USA, p 1141.
- Grunt S., Fieggen A.G., Vermeulen R.J., Becher J.G., Langerak N.G., 2014. Selection criteria for selective dorsal rhizotomy in children with spastic cerebral palsy: a systematic review of the literature. *Developmental Medicine & Child Neurology* 56, 302-312.
- Gump W.C., Mutchnick I.S., Moriarty T.M., 2013. Selective dorsal rhizotomy for spasticity not associated with cerebral palsy: reconsideration of surgical inclusion criteria. *Neurosurgical Focus* 35, E6.
- Hurov L., 1985. Laminectomy for cauda equina syndrome in a cat. *Journal of the American Veterinary Medical Association* 186, 504-505.
- McLeod W.M., Trotter D.M., Lumb J.W., 1958. *Bovine Anatomy*. 2ed., Burgess Publishing Company, Minneapolis, Minnesota, USA, p 232.
- Pavaux C., Sautet J., Lignereux J.Y., 1985. Anatomy of the bovine gastrocnemius muscle as application to the surgical correction of spastic paresis. *Vlaams Diergeneeskundig Tijdschrift* 54, 296-312.
- Ranck J.B., 1975. Which elements are excited in electrical stimulation of mammalian central nervous system: a review. *Brain Research* 98, 417-440.
- Roberts A., 2013. Surgical management of spasticity. *Journal of Children's Orthopaedics* 7, 389-394.
- Seiferle E., 1992. In: Nickel, R., Schummer A., Seiferle E.: *Lehrbuch der Anatomie der Haustiere*. 4. Nervensystem, Sinnesorgane, endocrine Drüsen, 3ed., Verlag Paul Parey, Germany, 1992, pp 39.

Selective dorsal rhizotomy to tackle quadriceps femoris muscle spasticity in cattle: pilot study

Steinbok P., 2006. Selection of treatment modalities in children with spastic cerebral palsy. *Neurosurgical Focus* 21, 1-8.

Van Schie P.E.M., Vermeulen R.J., van Ouwerkerk W.J.R., Kwakkel G., Becher J.G., 2005. Selective dorsal rhizotomy in cerebral palsy to improve functional abilities: evaluation of criteria for selection. *Child's nervous system* 21, 451-457.

Vaughan C.L., Subramanian N., Busse M.E., 1998. Selective dorsal rhizotomy as a treatment option for children with spastic cerebral palsy. *Gait & Posture* 8, 43-59.

Vlaminck L., De Moor A., Martens A., Steenhaut M., Gasthuys F., Desmet P., Van Branteghem L., 2000. Partial tibial neurectomy in 113 Belgian blue calves with spastic paresis. *Veterinary Record* 147, 16-19.

Wheeler S.J., Sharp N.J.H., Savage W. P., Trumpey J.E., 2005. *Small animal spinal disorders: diagnosis and surgery*. 1ed., Elsevier Mosby, St. Louis, Minnesota, USA, p 379.

CHAPTER 6

General discussion

Because in the last ten years, a higher proportion of animals with an atypical entity of spastic paresis was presented to the large animal clinic of the Faculty of Veterinary Medicine at Ghent University, veterinary surgeons were faced with difficulties how to differentiate those cases from the more frequently encountered gastrocnemius spasticity cases. Moreover, no scientific data were available on surgical outcome using selective tibial neurectomy in atypical cases, while an efficient alternative surgical treatment seemed not readily available. Therefore, this PhD study aimed at enhancing the diagnostics and management of calves affected with an atypical entity of spastic paresis and at developing an experimental surgical procedure to treat spasticity involving the quadriceps femoris muscle.

In the following section the main accomplishments of this PhD research will be discussed in a broader context with special attention to opportunities for future research.

In the introductory chapter to this PhD thesis, the existing knowledge on the epidemiology, pathogenesis, aetiology, diagnosis and treatment of spastic paresis was reviewed. Many topics mentioned in **Chapter 1** concerning this complex disease (e.g. pathogenesis, aetiology) remain to be elucidated and may be interesting pathways for future research.

When looking at the research on the pathogenesis of BSP, a lot of work has been done to reveal structural defects in spastic calves, but the few observed structural anomalies, such as degenerative lesions in certain areas of the central nervous system, were not consistently present in the affected animals (Chomiak and Szteyn, 1970; Milart and Chomiak, 1971; Chomiak et al., 1972; Baird et al., 1974; Dewulf et al., 1982). Possibly, spastic paresis in cattle is rather the result of a functional deficit than a structural lesion, e.g. a focal anomaly in the intercellular communication. Indeed, neurotransmitters play an important communicative role in the nervous system, and as a consequence, future investigation on functional abnormalities of their receptors may be appropriate. By means of immunohistochemical techniques, specific neurotransmitter receptors in the nervous system may be quantified and the results may contribute to a better understanding of the pathogenesis of the disease.

However, researchers will be faced with two main challenges. First of all, it is unknown which region of the nervous system should be investigated by immunohistochemistry as there is still no scientific consensus whether the defect is located at a central or peripheral level. Secondly, commercially available anti-neurotransmitter antibodies for immunohistochemistry that have a proven reactivity in cattle are very scarce. As a consequence, the species reactivity of the antibodies will first have to be tested in calves before they can be applied to examine bovine neural tissues and produce reliable results.

To date, the genetic component of spastic paresis has not been identified. Quadriceps femoris muscle involvement in spastic paresis is only described in two breeds, in Belgian Blues and in Romagnola cattle (Touati et al., 2003; Gentile et al., 2006). Both breeds have an abundant muscle development and a rapid growth rate. Moreover, the spastic calves of the retrospective study mainly had a superior muscularity. Therefore, an association between spastic paresis and a mutation in the region of the bovine growth hormone gene or myostatin gene may be possible.

The exact aetiology of BSP remains unknown despite the efforts of many researchers. However, it is generally accepted that some calves have an inherited predisposition to spastic paresis, which only becomes clinically observable in the presence of certain unidentified environmental, nutritional, metabolic or other conditions (Dawson, 1975; Van Huffel et al., 1986). A lot of research has been done to identify the triggering conditions for the phenotypical expression of spastic paresis, without success (Stegenga, 1964; De Ley and De Moor, 1977; Arnault, 1979; Arnault, 1982; Van Huffel et al., 1986; Ledoux, 2001; Ledoux, 2004).

From a genetic point of view, spastic paresis has to be eradicated and treatment of BSP animals is questionable. In Switzerland, veterinarians refuse to perform any surgical intervention on calves suffering from bovine spastic paresis (Karl Nuss, personal communication; Adrian Steiner, personal communication). Respective farmers are advised to bring affected animals to slaughter. Affected animals and carriers should be banned from breeding purposes in any case. Affected animals can easily be identified, as the clinical signs are very characteristic for the disease. However, a reliable tool to identify carriers of

spastic paresis does not exist. Straight hocks are suggested to be an indicator for carrier animals as they are often linked to it, but further research is necessary to confirm or invalidate this statement (Coopman et al., 2000). Coopman et al. (2000) stated that eradication of the spastic paresis trait should be possible by implementation of a molecular diagnostic test. This would enable testing the carrier status of animals before breeding to prevent the crossing of two carriers. However to date, such a test is not yet available. The challenging first steps towards a test would include the identification of possible markers or possible candidate genes. Because spastic paresis may have a heterogeneous genetic background, even within one breed, several different molecular tests may be needed to trace carrier animals (Hanset et al., 1993).

The results of the retrospective analysis presented in **Chapter 3** demonstrate that each type of BSP demands a different approach. In BSP-G cattle, surgical treatment options such as triple tenectomy or (selective) tibial neurectomy have been described with success rates of more than 80%, but based on the results of our recent study, it was concluded that these success rates cannot be extrapolated to the other two clinical entities of spastic paresis (Bouckaert and De Moor, 1966; Weaver, 1991; Vlaminck et al., 2000; Barvalia and Patil, 2003). None of the presented BSP-Q animals was surgically treated. This decision was based on the follow-up data of a few BSP-Q cases accidentally treated by selective neurectomy of the tibial nerve (cases date from before this PhD study and are not included in the present data). These animals were unable to rise following surgery due to a combination of insufficient strength to extend the tarsocrural joint (gastrocnemius muscle denervation) and continuous spasticity of the quadriceps femoris muscle. They were observed to remain in a dog sitting posture and this condition resulted in early slaughter (Lieven Vlaminck, personal communication). However, BSP-M cases with a minor involvement of the quadriceps femoris muscle might be considered for selective tibial neurectomy to enhance their quality of life and enable further weight gain. Moreover, all conservatively managed BSP-M cases in this study were prematurely culled due to worsening of their clinical condition.

A minority of conservatively managed animals with clinical signs of BSP-Q in the present study demonstrated spontaneous improvement of their spasticity, which prevented early culling due to insufficient weight gain, the main problem observed in the majority of these animals. Spontaneous improvement of spasticity in the untreated limb of bilaterally BSP-G affected animals that were unilaterally operated, was also observed in this study. Although no explanation can be given for this evolution, these observations imply that decisions on premature culling of affected animals should not be taken too rapidly. Our research was not able to identify time delays or ages of onset when the first signs of amelioration of clinical signs were observed. Neither were we able to determine whether clinical signs first worsened before decreasing in intensity. Close and repetitive monitoring of conservatively managed BSP-Q animals over longer time periods might enable to determine how long (time since observation of first clinical signs; absolute age of the animal) can be waited for improvement before deciding to cull.

The follow-up modality is the main limitation of the study of Chapter 3. The author did not observe the condition of the calves during the follow-up period, as the long-term results were based on the information of the farmer by means of a telephone questionnaire. Moreover, the judgement of the farmer might be an important source of bias in this study. Therefore, the outcome of the present study should be interpreted cautiously.

To identify involvement of the quadriceps femoris muscle in calves showing clinical signs of spastic paresis, three different techniques for femoral nerve blocking were developed and evaluated in cadavers in **Chapter 4.1**.

The dorsal paravertebral approach was the most user-friendly technique for femoral nerve blocking in the cadaver study and for this reason it was evaluated in the clinical trial. However, the other two techniques may also be used in living animals. As demonstrated in the clinical trial, the loss of skin sensitivity in the inner thigh is a good parameter to check the accuracy of the block. An unsuccessful block can be repeated regardless of the technique that was used.

Re et al. (2014) evaluated an ilial approach for performing regional anaesthesia of the femoral nerve both in a cadaver study and a clinical trial. Their intended clinical use was to provide analgesia during surgery of the hind limb. These authors targeted the same region of the femoral nerve as we did (Chapter 4.1), but the femoral nerve itself was visualized ultrasonographically, whereas we did not visualize the femoral nerve but determined its location based on landmarks (femoral artery and vein). A lidocaine 2% and epinephrine (0.002%) mixture was used, whereas in our study a procaine 4% solution was injected and the volume of local anaesthetics that was injected was higher than in our study (0.2 ml/kg vs. 0.1 ml/kg). The injections were performed in the sedated standing animal, and sedation was reversed after injection with 0.01 mg/kg atipamezole IV. Re et al. (2014) achieved a higher accuracy in the cadaver study in comparison to the results reported in Chapter 4.1 (6/10 vs. 8/20). However, in the clinical trial of Re et al. (2014), a variable degree of analgesia was produced after femoral nerve blocking in the standing animal and a high proportion of calves fell down within 15 minutes following unilateral administration of the local anaesthetic. A hypothesis for this observation was not made. Perhaps, because of the use of a larger volume, the local anaesthetics diffused towards the spinal cord, making the animal unable to bear weight on the hind limbs.

A femoral nerve block was experimentally performed in two sound calves in our institution using the ilial approach as described in the cadaver study (Chapter 4.1), but paralysis of the femoral nerve was not evoked following the blocks (data not included in this PhD study). Proper ultrasonographic identification of the femoral artery and vein, which are the landmarks for the ilial approach, and unwanted movements of the calves while advancing the needle were the main encountered difficulties.

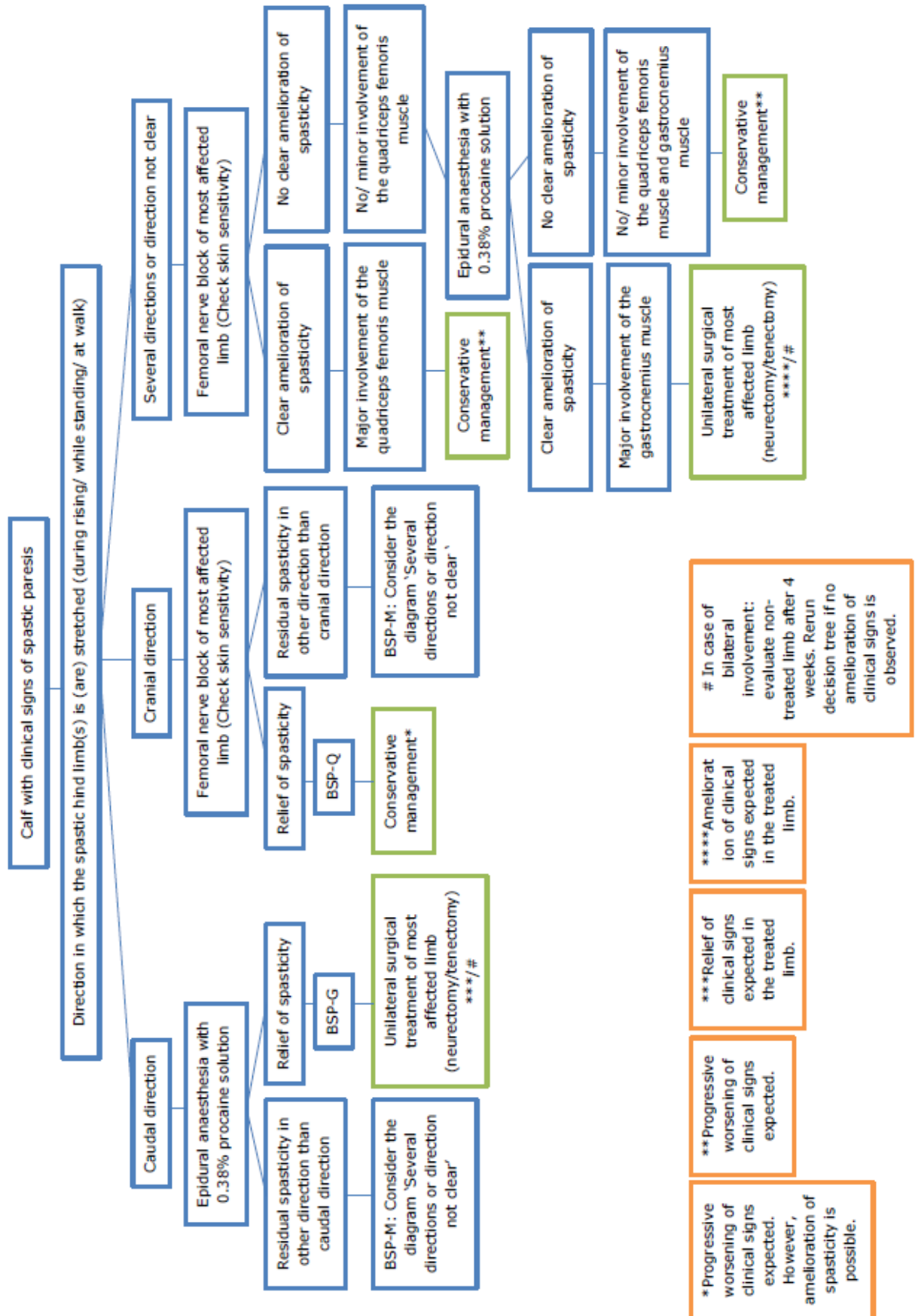
The results of the clinical anaesthesia trial of the femoral nerve (**Chapter 4.2**) clearly demonstrated the convenience and efficacy of the dorsal paravertebral approach in both sound and affected animals. The main clinical conclusion that can be drawn from this study was that this technique can be used not only to determine the involvement of the quadriceps femoris muscle, but that the

evaluation of the ambulation after anaesthesia can assist the decision whether to perform selective tibial neurectomy or not (Table 12).

Table 12 - Interpretation of spasticity following a femoral nerve block with respect to the implementation of selective tibial neurectomy in the treatment of bovine spastic paresis.

Involved muscle	Direction of spasticity	Result of femoral nerve block	Partial tibial neurectomy
Gastrocnemius muscle	caudal	No loss of spasticity	advised
Quadriceps femoris muscle	cranial	Total loss of spasticity	not advised
Several muscles, not clear which muscle group is dominant	unclear	Loss of spasticity +++ Loss of spasticity +	not advised advised

When combining the knowledge obtained in the previous chapters of this thesis, the following flowchart can be used to decide about calves suffering from spastic paresis.



* Progressive worsening of clinical signs expected. However, amelioration of spasticity is possible.

** Progressive worsening of clinical signs expected.

*** Relief of clinical signs expected in the treated limb.

**** Amelioration of clinical signs expected in the treated limb.

In case of bilateral involvement: evaluate non-treated limb after 4 weeks. Rerun decision tree if no amelioration of clinical signs is observed.

The bodyweight of a patient, however, may limit the success of a femoral nerve block. Firstly, fixating a heavy animal in lateral recumbency without the use of a sedative might be a huge challenge for both the operator and the animal. Forceful handling may induce high stress levels, which are very harmful to Belgian Blues as they have a reduced stress tolerance (Arthur, 1995). Moreover, wounds and bone fractures are more likely to occur when handling heavy animals. Secondly, the femoral nerve is much deeper located in heavy animals than in smaller ones, and is therefore much more difficult to approach. Furthermore, as a prognostic tool, femoral nerve blocks may be irrelevant in heavy animals as these animals are not ideal candidates for selective tibial neurectomy because their weight makes them more susceptible to develop postoperative gastrocnemius muscle rupture.

In BSP-M cases several muscle groups are involved in the spasticity, but a method to identify other spastic muscle groups besides the quadriceps femoris or gastrocnemius muscle is lacking. Hence, additional nerve blocks may be developed to identify spasticity of these other muscle groups. The gluteal muscles form one of the major muscle groups of the hindquarters and are therefore most interesting. To identify gluteobiceps spasticity, a caudal gluteal nerve block may be developed. Moreover, the access to this nerve to perform the block seems rather simple. However, developing a technique to block the rami musculares of the sciatic nerve, which are also innervating the gluteobiceps muscle, will be more challenging. The approach to the cranial gluteal nerve, which innervates the middle, deep, and accessory gluteal muscles and tensor fasciae latae muscle, seems less easy as it has a rather deep location and is surrounded by blood vessels (Budras and Habel, 2003). However, further research is necessary to evaluate different approaches for these nerves and to test these nerve blocks in living animals.

The ethical and financial aspects of the disease were the motivations for this research on the diagnosis and treatment options for BSP-calves. Using the diagnostic protocol developed during this research, calves can be identified as 'suitable for classic surgery or 'not suitable for classic surgery' and as a consequence, unfavourable surgical (and financial) outcomes can be minimized. Besides surgery in specific cases, farmers have to decide between following options for their affected animals: conservative management, premature

slaughter or euthanasia. When looking at the situation in our country, Belgian Blue calves are very valuable. Euthanasia or premature slaughter of all BSP-calves would implement an important economic loss for the farmer. Moreover, the disease is known to be painful. Therefore, keeping affected animals with worsening of clinical signs untreated for a longer period is unacceptable from an ethical point of view. For these reasons, research on new treatment options for BSP-calves suffering from an atypical presentation of the disease, as described in Chapter 5, is justified.

Prospecting the objective evaluation of the efficacy of dorsal rhizotomy to treat quadriceps femoris muscle spasticity, a technique to record cord dorsum potentials in calves was developed (**Chapter 5.1**). As the procedure of cord dorsum potential recordings with saphenous nerve stimulation was standardized during our work, cord dorsum potentials evaluation would be an important adjunct of peripheral sensory nerve testing to accurately assess the functional severity and distribution of abnormalities in proximal sensory/ mixed nerves, in dorsal nerve roots and in the spinal cord dorsal horns of calves. CDPs would be beneficial especially in animals with suspected neuropathy, radiculopathy or nerve root injury, or myelopathy involving the lumbosacral intumescences.

In **Chapter 5.2**, a surgical method to tackle quadriceps femoris muscle spasticity was evaluated in both sound and spastic calves. As the quadriceps femoris muscle activity is indispensable for retaining a standing posture in cattle, reducing spasticity without inducing paralysis of the affected muscle is essential in the development of a treatment for quadriceps femoris muscle spasticity. Consequently neurodestructive techniques such as a chemical neurolytic block or neurectomy are contra-indicated in the treatment of quadriceps femoris muscle spasticity.

Therefore, selective dorsal rhizotomy, performed for deafferentation of the quadriceps femoris muscle, seems a more appropriate treatment to tackle quadriceps femoris muscle spasticity as it does not affect its motor function. This technique was unilaterally evaluated in two sound calves and two calves suffering from bilateral bovine spastic paresis with quadriceps femoris muscle involvement. Correct identification of neural segments in the caudal part of the

lumbar spine was the most difficult step in the procedure. Autopsy revealed that in none of the four calves a complete deafferentation of the quadriceps femoris muscle had been performed. Further research is necessary to establish firm guidelines that enable precise preoperative localization of relevant neural segments. In literature, topography of neural segments in adult cattle is described relative to the vertebral bodies (Seiferle, 1992; Barone, 2010). However, during laminectomy a dorsal approach via the spinous processes and vertebral arches to the spinal cord is performed and these structures are not located perpendicular to the vertebral bodies, making the topographic description of neural segments in literature ineffective as a base for the approach to the neural segments of interest during surgery. Moreover, in our study calves of different ages and breeds were used in which the ascent of the spinal cord was still progressing, whereas in literature descriptions of the topography of neural segments in growing cattle are lacking. Therefore, evaluating the position of the neural segments relative to the spinous processes and vertebral arches in calves of different ages and different breeds is necessary to approach accurately the spinal cord for selective dorsal rhizotomy in young animals.

In the BSP-M calf, surgery was not successful. Several factors may explain this result. Firstly, lack of residual voluntary muscle strength of the quadriceps femoris muscle might have caused the gait deterioration, coordination disturbances and weakness of the hindquarters after surgery. Secondly, the calf might have suffered from a supplementary disorder resulting in increased muscle tone in which the stretch reflex loop is not affected such as dystonia. Unfortunately, to date, no objective tests exist in animals to assess the function of the different muscle groups that can be involved in spastic paresis or to identify dystonia in calves. Therefore, selection of suitable candidates for rhizotomy is a subject for improvement. The sole selection parameters for rhizotomy in this research study were the presence of quadriceps femoris muscle spasticity and the body weight of the calves, the latter because manual support during postoperative walking would be more difficult in heavy animals. However, the strength of antigravitational muscles of the hindquarters and the presence of other neurological deficits seem also important selection parameters. In children, prospective candidates for rhizotomy are evaluated thoroughly by a multidisciplinary team that consists of a pediatric neurologist, a pediatric

neurosurgeon, an orthopedic surgeon, a pediatric psychologist, a pediatric physiotherapist and occupational therapists (Vaughan et al., 1998). For these patients, preoperative tests, one of which is to evaluate the strength of different muscles, have been developed before deciding whether the surgery is performed or not. Muscle strength of antigravitational muscles of the lower limbs can be evaluated by the Manual Muscle Testing Scale or can be measured with a handheld dynamometer (Berry et al., 2004; Cole et al., 2007). Children with weakness of antigravitational muscles are excluded for surgery as a postoperative diminution of functional skills is seen in these patients (Steinbok, 2006). However, for both tests, voluntary cooperation of the patient is required, excluding these tests for use in veterinary medicine. Unfortunately, there are no objective preoperative tests available to assess the antigravitational strength of the different muscle groups in animals that can be involved in atypical spastic paresis cases or to identify the presence of other neuromuscular disorders such as dystonia, making it impossible to exclude animals suspected of postoperative weakness of the hindquarters for selective dorsal rhizotomy.

Further research is required to optimise the postoperative evaluation of the success of a selective dorsal rhizotomy. To evaluate the success of the procedure, posture and gait of the spastic calves in our study have been visually assessed. However, this method is rather subjective and incomplete in describing the postoperative change in neuromuscular functioning of these patients. In children, tests such as the gross motor function measure are designed to monitor different activities (lying, rolling, sitting, crawling, kneeling, standing, walking, running and jumping) that are important in a child's motor development (Vaughan et al., 1998; Funk et al., 2015). By performing these tests, the functional gain is more extensively examined. Specialized equipment is not required for these tests, but unfortunately the cooperation of the subject is again necessary to perform them, making it difficult to introduce this in veterinary medicine. Measurement of temporal-distance parameters provides a more objective way to evaluate gait changes. To evaluate the gait pre- and post-operatively in spastic animals, stride length, stride time and step overlap may be interesting parameters. They may be deduced from gait analysis using the pressure plate in spastic calves. As automatic lameness detection is a hot topic in research on dairy cattle, the use of pressure plates has already been

experimentally introduced in cattle (van der Tol et al., 2002; van der Tol et al., 2003; Walker et al., 2010; Maertens et al., 2011). However, further research is required to evaluate the use of pressure plates for the measurement of quantitative improvement of gait in spastic calves following a rhizotomy procedure.

A further modification of the technique of selective dorsal rhizotomy is necessary before standardized use in patients. Therefore, the technique used in our study has to be compared with other reports for refinement. Selective dorsal rhizotomy has been described in three calves suffering from BSP-G, but a detailed description of the rhizotomy technique was lacking in this report (De Ley and De Moor, 1977). In contrast, descriptions of the technique are numerous in human medicine, and are slightly different from our technique. First of all, in children, selective dorsal rhizotomy to treat spasticity in the lower limbs is focused on the neural segments of L1-S2, which are located in the cauda equina, whereas in our study, surgery was performed on the level of the lumbar spinal cord (Park et al., 1993; Farmer and McNeely, 2005; Abbott, 2009; Mertens, 2014). By performing surgery in the cauda equina, a small laminectomy window is sufficient to expose all dorsal roots of interest, which is in contrast with the situation in cattle. Secondly, in children the dorsal roots of the L2-S2 neural segments are electrically stimulated and those roots giving an abnormal EMG response are isolated. These 'abnormal' roots cause either an evoked muscle activity outside of its myotome or activity lasting after cessation of the stimulus. Then, each rootlet of the abnormal roots is stimulated, and those rootlets resulting in abnormal EMG responses are transected (Farmer and McNeely, 2005; Abbott, 2009). In our study, however, abnormal EMG responses were not observed which made it impossible to discriminate rootlets contributing in spasticity from normal rootlets. As a consequence, there was aimed at transecting all dorsal rootlets showing reciprocity with the quadriceps femoris muscle. Finally, an operating microscope is used for precise EMG testing and sectioning of the dorsal roots when rhizotomy in children is performed (Park et al., 1993). Also in calves, it is felt that the use of an operating microscope might facilitate visualization, accurate stimulation and handling of the tiny dorsal rootlets.

Based on the results of this PhD thesis, it can firstly be concluded that selective tibial neurectomy is very successful in BSP-G calves and results in improvement of the clinical signs in BSP-M calves with a minor involvement of the quadriceps femoris muscle. In non-treated BSP-M calves, worsening of clinical signs is expected and in BSP-Q cases, worsening of clinical signs is most frequently seen, but spontaneous improvement is possible. Secondly, it can be stated that the femoral nerve block, by performing a dorsal paravertebral approach, is an objective diagnostic tool to identify quadriceps femoris muscle spasticity in BSP-M and BSP-Q calves. Moreover, in BSP-M calves, the results of an femoral nerve block can be used as a prognostic tool prior to selective tibial neurectomy. Thirdly, selective dorsal rhizotomy does not cause permanent locomotor problems in sound calves, but further modifications of the procedure are necessary before it may be used to tackle quadriceps femoris muscle spasticity. Overall, the developed techniques of this PhD thesis may be useful for future clinical applications or experimental purposes in cattle.

References

- Abbott R., 2009. Selective Dorsal Rhizotomy for the Treatment of Childhood Spasticity. In: Practical Handbook of Neurosurgery From Leading Neurosurgeons, 1ed., Springer-Verlag/Wien, Mörlenbach, Germany, pp. 387-397.
- Arnault G., 1979. Etude de la parésie spastique des bovins. Rivation et Cie, Bobigny, France.
- Arnault G., 1982. Bovine spastic paresis. An epidemiological, clinical and therapeutic study in a Charolese practice in France. Efficacy of Lithium Therapy. Proceedings of the XIIth World Congress of Diseases of Cattle, The Netherlands, Amsterdam, 853-858.
- Arthur P.F., 1995. Double muscling in cattle: A review. Australian Journal of Agricultural Research 46, 1493-1515.
- Baird J.D., Johnston K.G., Hartley W.J., 1974. Spastic paresis in friesian calves. Australian Veterinary Journal 50, 239-245.
- Barone R., 2010. Anatomie comparée des mammifères domestiques, Vol.7 : Neurologie II : Vol.7 : Neurologie. Editions Vigot, Paris, France, p 161.
- Barvalia D.R., Patil D.B., 2003. Modified gastrocnemius tenectomy with intact deep calcaneal tendinous tissues to relieve spastic paresis in bullocks. Veterinarski Arhiv 73, 295-300.
- Berry E.T., Giuliani C.A., Damiano D.L., 2004. Intrasession and intersession reliability of handheld dynamometry in children with cerebral palsy. Pediatric Physical Therapy 16, 191-198.
- Bouckaert J.H., De Moor A., 1966. Treatment of spastic paralysis in cattle: improved de-nerivation technique of the gastrocnemius muscle and post-operative course. Veterinary Record 79, 226-229.
- Budras K., Habel R.E., 2003. Bovine Anatomy. An illustrated Text. Schlütersche, Hannover, pp 17.

- Chomiak M., Szteyn S., 1970. Veränderungen im zentralen Nervensystem bei einem Fall von spastischer Parese des Rindes. Schweizer Archiv für Tierheilkunde 112, 397-401.
- Chomiak M., Lakomy M., Szteyn S., Milart Z., 1972. Weitere histopathologische Untersuchungen des Zentralnervensystems bei der Paresis spastica des Rindes. Archiv für experimentelle Veterinärmedizin 27, 791-798.
- Cole G.F., Farmer S.E., Roberts A., Stewart C., Patrick J.H., 2007. Selective dorsal rhizotomy for children with cerebral palsy: the Oswestry experience. Archives of Disease in Childhood 92, 781-785.
- Coopman F., Peelman L., Van Zeveren A., 2000. Overerving van spastische parese, normale en afwijkende spronghoeken en hun onderlinge verband in het belgisch witblauw ras: een kritische benadering. Vlaams Diergeneeskundig Tijdschrift 69, 249-257.
- De Ley G., De Moor A., 1977. Bovine spastic paresis: results of surgical deafferentation of the gastrocnemius muscle by means of spinal dorsal root resection. American Journal of Veterinary Research 38, 1899-1900.
- Dewulf M., De Moor A., Cockelbergh D., 1982. Spastische Parese bij het rund: Vergelijkende studie van het gehalte aan dopamine en noradrenaline in verschillende hersengebieden. Vlaams Diergeneeskundig Tijdschrift 51, 433-437.
- Farmer J.-P., McNeely P.D., 2005. Surgery in the Dorsal Roots for Children with Cerebral Palsy. Operative Techniques in Neurosurgery 7, 153-156.
- Funk J.F., Pathen A., Bakir M.S., Gruschke F., Sarpong A., Wagner C., Lebek S., Haberl E.J., 2015. Predictors for the benefit of selective dorsal rhizotomy. Research in Developmental Disabilities 37, 127-134.
- Gentile A., Testoni S., 2006. Inherited disorders of cattle: a selected review. Slovenian Veterinary Research 43, 17-29.
- Hanset R., Serteyn D., Grobet L., Michaux C., 1993. The bovine spastic paresis and its inheritance. Annales de Médecine Veterinaire 137, 237-247.

General discussion

- Ledoux J.-M., 2001. Bovine spastic paresis: etiological hypotheses. *Medical Hypotheses* 57, 573-579.
- Ledoux J.-M., 2004. Hypothesis of interference to superinfection between bovine spastic paresis and bovine spongiform encephalopathy; suggestions for experimentation, theoretical and practical interest. *Medical Hypotheses* 62, 346-353.
- Maertens W., Vangeyte J., Baert J., Jantuan A., Mertens K.C., De Campeneere S., Pluk A., Opsomer G., Van Weyenberg S., Van Nuffel A., 2011. Development of a real time cow gait tracking and analysing tool to assess lameness using a pressure sensitive walkway: the GAITWISE system. *Biosystems Engineering* 110, 29-39.
- Mertens, P., 2014. Neurosurgery for Spasticity. In: *Neurosurgery for Spasticity. A Practical Guide for Treating Children and Adults*, 1ed., Springer (ebook), pp365-386.
- Milart Z., Chomiak M., 1971. Pathological Changes in the Central Nervous System of a Bull Affected with Spastic Paresis. *The Veterinary Record* 88, 66-68.
- Park T.S., Gaffney P.E., Kaufman B.A., Molleston M.C., 1993. Selective lumbosacral dorsal rhizotomy immediately caudal to the conus medullaris for cerebral palsy spasticity. *Neurosurgery* 33, 929-933.
- Re M., Blanco-Murcia J., Villaescusa Fernández A., De Gaspar Simón I., Gómez de Segura I.A., 2014. Ultrasound-guided anaesthetic blockade of the pelvic limb in calves. *The Veterinary Journal* 200, 434-439.
- Seiferle E., 1992. In: Nickel, R., Schummer A., Seiferle E.: *Lehrbuch der Anatomie der Haustiere*. 4. Nervensystem, Sinnesorgane, endocrine Drüsen, 3ed., Verlag Paul Parey, Germany, 1992, pp 39.
- Stegenga T., 1964. Aangeboren afwijkingen bij rundvee. *Tijdschrift voor Diergeneeskunde* 89, 286-293.
- Steinbok P., 2006. Selection of treatment modalities in children with spastic cerebral palsy. *Neurosurgical Focus* 21, 1-8.

- Touati K., Muller Ph., Grulke S., Peters F., Serteyn D., 2003. La parésie spastique du quadriceps fémoral: Une nouvelle entité clinique chez le veau de race Blanc Bleu Belge. *Annales de Médecine Vétérinaire* 147, 261-265.
- van der Tol P.P.J., Metz J.H.M, Noordhuizen-Stassen E.N., Back W., Braam C.R., Weijs W.A., 2002. The Pressure Distribution Under the Bovine Claw During Square Standing on a Flat Substrate. *Journal of Dairy Science* 85, 1476-1481.
- van der Tol P.P.J., Metz J.H.M, Noordhuizen-Stassen E.N., Back W., Braam C.R., Weijs W.A., 2003. The vertical ground reaction force and the pressure distribution on the claws of dairy cows while walking on a flat substrate. *Journal of Dairy Science* 86, 2875-2883.
- Van Huffel X., De Moor A., Lampo P., 1986. Bovine spastic paresis - Genetic and environmental factors. *Vlaams Diergeneeskundig Tijdschrift* 55, 21-30.
- Vaughan C.L., Subramanian N., Busse M.E., 1998. Selective dorsal rhizotomy as a treatment option for children with spastic cerebral palsy. *Gait and Posture* 8, 43-59.
- Vlaminck L., De Moor A., Martens A., Steenhaut M., Gasthuys F., Desmet P., Van Branteghem L., 2000. Partial tibial neurectomy in 113 Belgian blue calves with spastic paresis. *Veterinary Record* 147, 16-19.
- Walker A.M., Pfau T., Channon A., Wilson A., 2010. Assessment of dairy cow locomotion in a commercial farm setting: The effects of walking speed on ground reaction forces and temporal and linear stride characteristics. *Research in Veterinary Science* 88, 179-187.
- Weaver A.D., 1991. Modified gastrocnemius tenectomy: a procedure to relieve spastic paresis in dairy calves. *Veterinary Medicine & Small Animal Clinician* 86, 1234-1239.

SUMMARY

Besides decreased animal welfare, bovine spastic paresis results in growth retardation and economic losses. The appearance of new clinical presentations of the disease since the turn of this century inspired the research performed in this thesis.

Even though researchers have spent decades trying to understand the aetiology and pathogenesis of the disease, much remains to be elucidated. Moreover, diagnosis and identification of spastic muscles still relies on subjective interpretations. This research is focused on objective identification and classification of various clinical presentations of the disease, formulation of a prognosis for each of these presentations as well as the development of a surgical treatment for calves suffering from quadriceps femoris muscle spasticity.

Chapter 1 introduces this PhD study by exposing the existing knowledge concerning bovine spastic paresis. It focuses on the clinical presentation of the disease as well as on the epidemiology. The pathogenesis and aetiology of the disease, which are still not totally elucidated, are described as well as the current diagnostic methods and treatment options for the classic presentation of spastic paresis. The lack of evidence-based classifications and the limited knowledge of the development and management of non-classic presentations of the disease provides a perfect rationale for the different studies that were performed.

The scientific aims of this work are presented in **Chapter 2**. The main aims were to evaluate the symptoms of different types of bovine spastic paresis (chapter 3), to develop an evidence-based diagnostic tool in order to identify involvement of the quadriceps femoris muscle (chapter 4) and to establish a surgical technique to tackle this quadriceps femoris muscle spasticity (chapter 5).

Chapter 3 reports the long-term clinical outcome in treated (selective tibial neurectomy) and non-treated calves suffering from bovine spastic paresis of the gastrocnemius muscle (BSP-G) or the quadriceps femoris muscle (BSP-Q) and with mixed muscle involvement (BSP-M) by means of a retrospective study. Medical records of 79 calves were analyzed and long-term follow-up information was obtained by a telephone questionnaire. The study group included 26 BSP-G (33%), 16 BSP-Q (20%) and 37 BSP-M (47%) cases. Twenty-five of the 26 BSP-G animals underwent surgery, which resulted in 86% of the cases in a complete resolution of clinical signs. Twenty-nine of the 37 BSP-M animals underwent surgery; none of them completely recovered but the majority improved (81.5%). Clinical signs gradually worsened in all the non-treated BSP-M animals. None of the BSP-Q calves were treated. In 2/3 of the BSP-Q calves the clinical signs gradually worsened whereas 1/3 of the animals improved spontaneously. It was concluded that selective tibial neurectomy is advocated for the treatment of BSP-G and in selected cases of BSP-M. However, in the latter group, this will only result in partial resolution of clinical signs. BSP-Q calves are not amenable to a selective tibial neurectomy but spontaneous improvement is possible.

In **Chapter 4** an evidence-based method to detect quadriceps femoris muscle spasticity is developed.

In **Section 4.1** an anaesthetic block of the femoral nerve is developed in cadavers, in order to identify quadriceps femoris muscle involvement in complex spastic paresis cases. Three different injection approaches of the femoral nerve (ventral paravertebral, dorsal paravertebral and ilial approach) were evaluated by simulated ultrasound-guided perineural injection of methylene blue in 10 cadavers. The study was preceded by detailed anatomical and cross-sectional investigation of relevant topography in 3 cadavers, to establish the best possible injection approaches. Ultrasound image quality, the number of needle redirections required for correct needle positioning, and the resultant injection success (injection score) were recorded. The dorsal paravertebral approach yielded the best results with 80% of targeted nerves properly stained post injection. It is concluded that this technique is preferred over others for use as a diagnostic tool in the differentiation of different spastic paresis entities.

The aim of the study in **Section 4.2** was to evaluate the clinical effects of a femoral nerve block via a dorsal paravertebral injection in healthy calves and calves suffering from spastic paresis. Based on bony landmarks and using ultrasound guidance, the femoral nerves of eight healthy calves were blocked bilaterally with a 4% procaine solution containing methylene blue dye. In 11/16 nerve blocks, paralysis of the quadriceps femoris muscle was obtained after dorsal paravertebral injection. Paralysis was total in 8/16 cases. The injection site was confirmed by post mortem dissection, and in 12/16 cases the blue dye was found < 2 mm from the nerve. Clinical use of the technique was then demonstrated in two cases of atypical bovine spastic paresis. In such calves an objective diagnostic tool is required to exclude calves with major involvement of the quadriceps femoris muscle from selective tibial neurectomy. The femoral nerve block used in this study has the potential to be such a method and can indeed be used to establish the involvement of the quadriceps femoris muscle in calves suffering from the quadriceps or mixed presentation forms of spastic paresis.

In **Chapter 5**, transection of dorsal nerve rootlets corresponding with the quadriceps femoris muscle is evaluated as a treatment option for quadriceps femoris muscle spasticity. To postoperatively check the integrity/destruction of dorsal rootlets showing reciprocity with the quadriceps femoris muscle, cord dorsum potentials were measured in the study in **Section 5.1**. Cord dorsum potentials (CDPs) are sensory evoked potentials that are being used to assess proximal sensory nerve, dorsal nerve root and spinal cord dorsal horn function. The CDP is a spinal cord field potential that arises in the region of the spinal cord segments receiving input from peripheral nerves. The purpose of the pilot study of this section was to establish normal values for CDP onset latency (OL), peak latency (PL) and peak-to-peak amplitude (PPA) after saphenous nerve stimulation. CDPs were recorded under general anaesthesia in 15 clinically and neurologically healthy calves. The saphenous nerve was stimulated with 2 monopolar needle electrodes. CDPs were recorded from the interarcuate space L3-L4, L4-L5, L5-L6 and L6-S1 with a monopolar needle electrode. OL (in ms) was measured as the shortest distance between the trigger point and the takeoff of the initial phase and PL (in ms) between the trigger point and the peak of the

Summary

highest phase. PPA (in mV) was measured between the two largest peaks of opposite polarity. CDPs were easily recorded at the different recording sites. CDPs consisted of a large negative peak (actual CDP) followed by a long latency positive phase; in some calves polarity was reversed. An initial small polyphasic wave was occasionally observed. It is concluded that CDPs in response to saphenous nerve stimulation can be reproducibly recorded in calves at L3-L4, L4-L5, L5-L6 and L6-LS1 recording sites, with the largest responses at L5-L6.

In **Section 5.2**, the technique of selective dorsal rhizotomy was evaluated as a treatment option for calves with quadriceps femoris muscle spasticity. Two sound calves and two calves suffering from bilateral hind limb spastic paresis with quadriceps femoris muscle involvement were used for this prospective study. All calves underwent a laminectomy and unilateral selective dorsal rhizotomy for deafferentation of the quadriceps femoris muscle. Pre- and postoperatively, the saphenous nerve was electrically stimulated and cord dorsum potentials were recorded. Rehabilitation of the calves was evaluated during one month. Autopsy was performed to verify the spinal nerve rootlets that were surgically destroyed and to locate the position of spinal cord segments of interest relative to the vertebrae. Following selective dorsal rhizotomy, cord dorsum potentials were absent in all operated limbs, except in one sound calf. After a short rehabilitation, both sound calves regained normal locomotor function. The gait of the first spastic calf improved as compared to the gait before surgery. However, in the second spastic calf, dorsal rhizotomy resulted in severe incoordination and weakness of the hindquarters. Autopsy revealed considerable variation in the location of the L3-L6 neural spinal cord segments in relation to the respective lumbar vertebrae. In conclusion we can state that in sound calves, the procedure of selective dorsal rhizotomy does not cause any permanent locomotor problem. In spastic calves, selective dorsal rhizotomy should be regarded as an experimental procedure for treatment. With more accurate identification of correct neural segments, the outcome of the procedure may be optimized.

The **final part** of this PhD thesis includes the general discussion and conclusions. All studies performed in the present PhD-thesis provided new insights in diagnostics, prognosis and treatment options for non-classic spastic paresis cases.

The major future challenges are the refinement of the selection procedure for rhizotomy candidates and the rhizotomy technique as well as fundamental topographic research on nerve roots and spinal cord segments in calves. Preventing spastic paresis is better than curing it. Therefore, advanced genetic research in this complex disease is advocated.

SAMENVATTING

Spastische parese bij runderen resulteert naast een verminderd dierenwelzijn in groeiachterstand, een lagere productie en bijhorende verminderde opbrengst. Het voorkomen van andere klinische presentaties van deze aandoening sedert de eeuwwisseling inspireerde het onderzoek uitgevoerd in het kader van deze thesis.

Hoewel er reeds veel onderzoek verricht werd naar deze ziekte, zijn er nog steeds grote lacunes in de literatuur betreffende het ontstaan en de ontwikkeling van spastische parese. Daarenboven gebeurt de diagnostiek en het identificeren van spastische spieren op subjectieve manier. De studie is gericht op het objectief classificeren van verschillende presentaties van de aandoening, op het formuleren van een wetenschappelijk onderbouwde prognose voor elk van de presentaties, evenals op het ontwikkelen van een chirurgische behandeling voor kalveren lijdend aan quadricepsspasticiteit.

Hoofdstuk 1 leidt het onderzoek in met het toelichten van de beschikbare kennis over spastische parese. Het spitst zich toe op de klinische presentatie van de aandoening met zijn 3 classificaties, evenals de epidemiologie. De weinig opgehelderde pathologie en etiologie, evenals de huidige diagnostiek en behandelingen voor de klassieke vorm van spastische parese worden beschreven. De wetenschappelijk weinig onderbouwde classificaties en het gebrek aan kennis over de evolutie en aanpak van de niet-klassieke presentaties illustreren de noodzaak van volgende onderzoeken.

De wetenschappelijke ambities van dit onderzoek worden voorgesteld in **Hoofdstuk 2**. De belangrijkste doelstellingen waren het objectief classificeren van de presentaties van de ziekte (hoofdstuk 3), de ontwikkeling van een wetenschappelijk onderbouwd diagnostisch hulpmiddel om spasticiteit van de quadricepsspier te identificeren (hoofdstuk 4) en het ontwikkelen van een

chirurgische techniek om spasticiteit van de quadricepsspier te behandelen bij kalveren (hoofdstuk 5).

Hoofdstuk 3 rapporteert de langetermijnresultaten bij behandelde en niet-behandelde kalveren die lijden aan spastische parese door middel van een retrospectieve studie. De analyse evalueerde de evolutie na klassieke ingreep bij dieren met gastrocnemiuspasticiteit (BSP-G) en gemengde spasticiteit (BSP-M) en na een conservatief management in geval van niet-klassieke presentaties nl. gemengde spasticiteit en quadricepsspasticiteit (BSP-Q). De medische fiches van 79 kalveren werden geanalyseerd en langetermijninformatie werd verkregen via een telefonische enquête. De studiegroep bevatte 26 BSP-G (33%), 16 BSP-Q (20%) en 37 BSP-M (47%) kalveren. Vijfentwintig van de 26 BSP-G dieren ondergingen chirurgie. Dit resulteerde in 86% van de gevallen in een volledig herstel. Negenentwintig van de 37 BSP-M dieren werden geopereerd. Geen van hen herstelde volledig, maar de meerderheid vertoonde een verbetering van de klinische tekens (81.5%). In alle niet-behandelde BSP-M kalveren verslechterden de klinische tekens geleidelijk aan. Geen enkele van de BSP-Q kalveren werd behandeld. Bij 2/3 van de BSP-Q kalveren werd een verergering van de klinische tekens vastgesteld, terwijl 1/3 van de dieren spontaan verbeterde. Er werd geconcludeerd dat een selectieve neurectomie van de n. tibialis aangewezen is als behandeling voor BSP-G dieren en voor specifieke BSP-M gevallen. In de laatste groep zal deze ingreep resulteren in een gedeeltelijke verdwijning van de klinische tekens. Conservatieve aanpak bij niet-klassieke spasticiteit resulteert doorgaans in verergering van de symptomen, hoewel spontane verbetering bij quadricepsspasticiteit mogelijk is.

In **Hoofdstuk 4** wordt een objectieve methode ontwikkeld om spasticiteit van de quadricepsspier te onderkennen. Op basis hiervan worden 3 klinische presentaties van spastische parese objectief gedifferentieerd, namelijk spastische parese van de gastrocnemiuspier (BSP-G), spastische parese van de quadricepsspier (BSP-Q) en een gemengde vorm van spastische parese (BSP-M).

Sectie 4.1 beschrijft een kadaverstudie waarin een punctietechniek voor de geleidingsanesthesie van de femoraliszenuw ontwikkeld wordt om betrokkenheid van de quadricepsspier te identificeren bij complexe spastische parese gevallen. Volgend op een grondige anatomisch-topografische studie van de achterhand van kalveren werden 3 verschillende benaderingswijzen voor het uitvoeren van die geleidingsanesthesie geëvalueerd (ventrale paravertebrale benadering, dorsale paravertebrale benadering, iliale benadering) door perineurale injecties met methyleenblauw in 10 kadavers uit te voeren. De echografische beeldkwaliteit, het aantal heroriënteringen van de naald vereist voor een correcte naaldpositie en het resulterende injectiesucces (injectiescore) werden vastgelegd. Met de dorsale paravertebrale benadering werden de beste resultaten behaald, waarbij tachtig procent van de zenuwen gekleurd was na injectie. Deze techniek wordt geprefereerd boven de andere om als diagnostisch hulpmiddel te gebruiken in de differentiatie tussen verschillende types van spastische parese.

In **Sectie 4.2** worden de klinische effecten van een geleidingsanesthesie van de n. femoralis door middel van de dorsale paravertebrale benadering geëvalueerd in gezonde kalveren en bij twee kalveren die lijden aan een niet-klassieke vorm van spastische parese. Gebaseerd op de beenderige oriëntatiepunten en met behulp van echografie werden de femoraliszenuwen bilateraal verdoofd bij 8 gezonde kalveren door middel van een 4% procaïne oplossing, die blauwe kleurstof bevatte. Een complete paralyse werd bekomen bij 8/16 gevallen. Tijdens autopsie werd de injectieplaats bevestigd en in 12/16 gevallen werd de blauwe kleurstof op minder dan 2 mm van de zenuw terug gevonden. De klinische toepassing van de techniek werd vervolgens gedemonstreerd in 2 kalveren met een atypische vorm van spastische parese. In zulke kalveren is een objectief diagnostisch hulpmiddel vereist om kalveren te selecteren die geschikt zijn om een selectieve neurectomie van de n. tibialis te ondergaan. De geleidingsanesthesie van deze studie heeft het potentieel om zo'n hulpmiddel te zijn en kan toegepast worden om betrokkenheid van de quadricepsspier aan te tonen in kalveren die lijden aan een quadricepsvorm of gemengde vorm van spastische parese.

In **Hoofdstuk 5** wordt het doorsnijden van de dorsale zenuwwortels die corresponderen met de quadricepsspier geëvalueerd als mogelijke behandeling voor quadricepsspasticiteit. Voor de postoperatieve controle van het al dan niet intact zijn van dorsale zenuwworteltjes die connectie met de quadricepsspier vertonen, worden in de studie van **Sectie 5.1** cord dorsum potentialen gemeten. Deze veldpotentialen van het ruggenmerg worden opgewekt door elektrische stimulatie van de n. saphenus, die de gevoelstak van de n. femoralis is. Het doel van de pilootstudie van deze sectie was om normaalwaarden voor CDP beginlatentie, pieklatentie en amplitude te bepalen na stimulatie van de n. saphenus. De CDPs werden gemeten in 15 klinisch en neurologisch gezonde dieren onder algemene anesthesie. De n. saphenus werd gestimuleerd met 2 monopolaire naaldelectrodes en de veldpotentialen werden gemeten in de ruimte tussen de wervelbogen van L3-L4, L4-L5, L5-L6 en L6-S1 met behulp van een monopolaire naaldelectrode. De beginlatentie (in ms) werd bepaald als de kortste afstand tussen het stimulatiepunt en de start van de initiële fase en de pieklatentie (in ms) tussen het stimulatiepunt en de piek van de hoogste fase. De amplitude (in mV) werd gemeten tussen de twee grootste pieken met omgekeerde polariteit. CDPs waren gemakkelijk te registreren ter hoogte van de verschillende meetplaatsen. CDPs bestonden uit een grote negatieve piek (de eigenlijk CDP), gevolgd door een lange latente positieve fase. In sommige kalveren was de polariteit omgekeerd. Occasioneel werd een initiële kleine polyfasische golf vastgesteld. Er kan geconcludeerd worden dat CDPs in antwoord op n. saphenus stimulatie reproduceerbaar kunnen gemeten worden in kalveren ter hoogte van de L3-L4, L4-L5, L5-L6 en L6-S1 meetplaatsen, met het grootste antwoord ter hoogte van L5-L6.

In **Sectie 5.2** wordt de selectieve rhizotomietechniek als behandelingsmogelijkheid voor kalveren met quadricepsspasticiteit geëvalueerd. De ingreep beschreven in de studie werd uitgevoerd op 2 gezonde kalveren en 2 kalveren die lijden aan bilaterale spastische parese waarbij de quadricepsspier betrokken was in de spasticiteit. Alle kalveren ondergingen een laminectomie en een eenzijdige selectieve dorsale rhizotomie met de-afferentatie van de quadricepsspier als doel. Voor en na operatie werd de n. saphenus elektrisch gestimuleerd en werden CDPs gemeten. Het herstel van de kalveren werd gedurende 1 maand opgevolgd. Vervolgens werd autopsie uitgevoerd om de

zenuwworteltjes die tijdens operatie vernietigd waren te verifiëren en om de positie van de lumbale ruggenmergsegmenten ten opzichte van de lendenwervels te bepalen. Na operatie konden geen cord dorsum potentialen geregistreerd worden bij stimulatie van de n. saphenus van de geopereerde zijde bij 3 van de 4 kalveren. Na een korte herstelperiode herwonnen de 2 gezonde kalveren vlot hun locomotorische vaardigheden. De gang van het eerste spastische kalf was verbeterd na operatie, maar bij het tweede spastische kalf leidde de dorsale rhizotomie tot erge incoördinatie en zwakte van de achterhand. Een duidelijke variatie in locatie van de ruggenmergsegmenten L3-L6 ten opzichte van de lumbaalwervels was zichtbaar tijdens autopsie. Er kan geconcludeerd worden dat de selectieve rhizotomieprocedure geen permanente locomotorische problemen veroorzaakt in gezonde kalveren. In spastische kalveren wordt de selectieve dorsale rhizotomie beschouwd als een experimentele behandelingsprocedure. Met een accuratere identificatie van de lumbale ruggenmergsegmenten zou het resultaat van de procedure geoptimaliseerd kunnen worden.

Het **laatste hoofdstuk** van dit proefschrift bevat de algemene discussie en conclusies. De verschillende studies van deze doctoraatsthesis hebben nieuwe inzichten opgeleverd in de diagnostiek, prognose en behandelingsmogelijkheden van spastische parese. De belangrijkste uitdagingen zijn momenteel het verfijnen van de selectieprocedure voor rhizotomiekandidaten en de rhizotomietechniek alsook een fundamenteel topografisch onderzoek omtrent zenuwwortels en ruggenmergsegmenten bij kalveren. Voorkomen is beter dan genezen, zo ook voor spastische parese. Geavanceerd genetisch onderzoek naar deze complexe ziekte is dan ook noodzakelijk.

CURRICULUM VITAE

Caroline De Vlaminck werd geboren op 3 september 1985 in Oostende. Na het beëindigen van het secundair onderwijs aan het Onze-Lieve-Vrouwecollege in Oostende (Wetenschappen-Wiskunde), startte zij in 2003 met de studie Diergeneeskunde aan de Universiteit Gent. Daar behaalde zij in 2009 het diploma van Dierenarts met grote onderscheiding.

Onmiddellijk na haar studies trad zij als voltijds assistent in dienst bij de Vakgroep Heelkunde en Anesthesie van de Huisdieren, dit onder de leiding van prof. Lieven Vlaminck. Haar voornaamste taak was de chirurgie bij herkauwers en tandheelkunde bij paarden. Voorts gaf ze klinisch onderricht aan studenten diergeneeskunde en nam deel aan de nacht- en weekenddiensten van de vakgroep. Daarnaast voerde ze wetenschappelijk onderzoek omtrent diagnostiek en management bij kalveren die lijden aan atypische presentaties van spastische parese. Tevens behaalde ze in 2015 het diploma van de doctoraatsopleiding in de diergeneeskundige wetenschappen.

Zij is auteur en mede-auteur van verschillende wetenschappelijke publicaties in internationale tijdschriften, was spreker op internationale congressen en reviewer voor 'Veterinary Record Case Reports' en 'Veterinary Anaesthesia and Analgesia'.

Publications

De Vlamynck C., Pille F., Hauspie S., Saunders J., Van der Stede Y., Gasthuys F., Vlaminck L., 2013. Evaluation of three approaches for performing ultrasonography-guided anesthetic blockade of the femoral nerve in calves. *American Journal of Veterinary Research* 74, 750-756.

De Vlamynck C., Vlaminck L., Hauspie S., Saunders J., Gasthuys F., 2013. Ultrasound-guided femoral nerve block as a diagnostic aid in demonstrating quadriceps involvement in bovine spastic paresis. *The Veterinary Journal* 196, 451-455.

Gunnarsdottir H., Van der Stede Y., **De Vlamynck C.,** Muurling F., De Clercq D., van Loon G., Vlaminck L., 2014. Hospital-based study of dental pathology and faecal particle size distribution in horses with large colon impaction. *The Veterinary Journal* 202, 153-156.

De Vlamynck C., Pille F., Vlaminck L., 2014. Bovine Spastic Paresis: Current Knowledge and Scientific Voids. *The Veterinary Journal* 202, 229-235.

De Vlamynck C., Pille F., Vlaminck L., 2015. Long-term outcome of conservative management or surgical treatment of bovine spastic paresis: 79 cases. *Veterinary Surgery*, in press.

Oral presentations and posters

Vlaminck L., **De Vlaminck C.**, Muurling F., Van der Stede Y., De Boever J., 2010.

Case control study to identify a possible relationship between dental pathology, impaction colic and faecal particle size distribution in horses: preliminary results. Proceedings of the 19th European Congress of Veterinary Dentistry, Nice, France, p.91-94.

De Vlaminck C., Vlaminck L., Hauspie S., Saunders J., Gasthuys F., 2013.

Ultrasound-guided femoral nerve block as a diagnostic aid in demonstrating quadriceps involvement in bovine spastic paresis. Lameness in Ruminants, 17th International symposium; 9th International conference, Abstracts., Bristol, United Kingdom, p.159-159.

De Vlaminck C., Vlaminck L., Hauspie S., Saunders J., Gasthuys F., 2013.

Ultrasound-guided femoral nerve block as a diagnostic aid in demonstrating quadriceps involvement in bovine spastic paresis. Proceedings European Buiatric Forum 2013, Marseille, France, p.99.

De Vlaminck C., Vlaminck L., Hauspie S., Saunders J., Gasthuys F., 2013.

Ultrasound-guided femoral nerve block as a diagnostic aid in demonstrating quadriceps involvement in bovine spastic paresis. World Veterinary Congress, 31st, Prague, Czech Republic, Abstracts.

Van Soens I., **De Vlaminck C.**, Pille F., Vlaminck L., Van Ham L., 2013.

Recording of cord dorsum potentials in calves. Neuro-emergency and critical care: ESVN and ECVN 26th symposium., Paris, France, p.75.

DANKWOORD

Hier is hij dan, het fameuze dankwoord, het meest gelezen stuk van 'Het Boekje'. Enerzijds keek ik er enorm naar uit om iedereen eens persoonlijk te kunnen bedanken die de afgelopen zes jaar direct of indirect meegeholpen heeft aan dit werk, anderzijds is het een ware uitdaging om niemand over te slaan.

Laat ik beginnen bij mijn promotoren. Lieven, bedankt om mij in te wijden in de runderchirurgie, de tandheelkunde en om me dit project toe te vertrouwen. Mijn ideeën over welke richting we zouden uitgaan in het onderzoek waren soms wat verschillend van de jouwe, maar je stond steeds voor mijn ideeën open en gaf me de vrijheid om mijn gekozen weg te bewandelen, waarvoor dank. Frederik, ik apprecieer je kritische reflectie en nuchtere kijk op de zaken in dit onderzoek enorm. Ook naast het doctoraat was het steeds een aangename samenwerking. Bedankt hiervoor. Iris, je kwam er als laatste bij, maar daarom was je bijdrage aan dit werk niet minder. Bedankt voor alle hulp en begeleiding in dit onderzoek, naast de leuke babbels tijdens de metingen uiteraard. Je bijdrage was een echte steun in de rug. Ik ben blij voor je dat je een nieuwe uitdaging gevonden hebt in het Antwerpse.

Professor Gasthuys, bedankt om me de kans te geven om van start te gaan binnen de vakgroep. Stijn Hauspie, bedankt voor de technische ondersteuning bij het echogebruik voor dit onderzoek. Hoe zou ik dit moeten geklaard hebben zonder jouw hulp? Ook Els, bedankt voor je hulp hierbij. Yves van der Stede en prof. Duchateau, bedankt voor jullie bijdrage in de statistiek. Prof. Luc Van Ham, bedankt voor uw inbreng bij de laatste proeven en uw kritische, maar constructieve opmerkingen. Dr. Tshamala, bedankt voor uw hulp en bereidwilligheid tijdens de neurochirurgieën. Annick Viaene, bedankt om een 'humaan' licht en inzicht te werpen op ons runderonderzoek. Het was een meerwaarde en leuk je er bij te hebben. Gunther Van Loon, bedankt voor je bereidwilligheid en motivatie bij de echogeleide iliale puncties. Het blijft jammer genoeg een mysterie waarom ze geen paralyse uitlokten. Pieter Cornillie,

Dankwoord

bedankt voor de begeleiding bij de stereometrie van de zebu en Christophe Casteleyn voor de dissectie van de zebu. Bart De Pauw, bedankt voor het maken van de foto's van mijn eerste publicatie. Ingrid, Casper en Kaatje, bedankt voor het maken van de CT-beelden van mijn kalfjes. Dr. Kevin De Cock, bedankt voor uw hulp op het moment dat we met wat vraagstukken zaten omtrent de cord dorsum potentialen. De dienst inwendige ziekten en farmacologie, en speciaal Elke Plessers, bedankt voor de aanlevering van de kalfjes. Christel Van Roy, bedankt voor de praktische tips om alles wat vlotter te laten lezen. Succes met je paarden! Bedankt aan alle studenten (de meesten ondertussen collega's) die meehielpen met het vasthouden, revalideren, zonnecrème smeren, medicatie toedienen... kortom alle zorgen van/voor/op mijn kalfjes! Zonder jullie hulp was dit onderzoek een stuk moeilijker uitvoerbaar geweest! Bedankt aan alle anesthesisten om de vaak langdurige gasanesthesieën tot een goed einde te brengen: Stijn, Miguel, Barbara, Tim, Sanne, Sofie, Diego, Ilaria, Daphné en Maarten, bedankt!

Professor Simoens, ik bewonder uw blijvende drive en passie voor uw vak. Het was heel motiverend en inspirerend om met u samen te werken. Ik apprecieer heel hard uw begeleiding, uw kritische vragen als 'advocaat van de duivel' en uw opmerkingen die u steeds op een constructieve manier aanbracht. U was onmisbaar tijdens het dissectiewerk en was immer bereid om mijn vragen te beantwoorden. Door uw bijdrage staan ook de puntjes op de i en zo heb ik het graag. Hartelijk bedankt voor alles!

Voorts wil ik de nog niet-vermelde leden van de lees- en examencommissie bedanken: Prof. Geert Opsomer, Prof. Kamal Touati en Dr. Bart Pardon, bedankt voor jullie bereidheid om in de jury te zetelen.

Naast de medewerkers van dit doctoraat wil ik ook mijn collega's en ex-collega's van de dienst Heelkunde bedanken voor de fijne tijd:

Ann, bedankt. Ik apprecieer je rechttoe rechtaan-aanpak. Stijn, ik was misschien niet bepaald tevreden toen ik plots ook anesthesiste werd, maar dat kwam eerder door de communicatie hierrond, dan door de job zelf. Bedankt voor alle goeie anesthesische tips die je me gaf. Sofie, ik ben blij dat je het goed hebt iets verder op de gang! Miguel, it was always nice to work with you, I hope you have a great time in Brasil. Barbara, I miss the time we were sharing the same office, it was always fun with you! Thank you for everything! Helga, I like your awesome attitude, you rock. Annelies, je oneliners blijven legendarisch. Ik ben blij dat je voldoening vindt in je (niet meer zo) nieuwe job. Het was een fijne tijd op 'den bureau'. Succes met de bouw en tot in den draai! Jeroen, ex-buurman, waar is de tijd van de biechtstoel? Bedankt voor alles wat ik van je leerde in de 'instelling'. Ik wens je veel geluk toe in de toekomst, je verdient het! Michèle, een top-orthopediste, een fijne collega en vriendin. Ik ben blij dat je het ware geluk gevonden hebt in Ruddervoorde met Fré en Emile. Ik hou dat poortje aan jullie tuin in mijn achterhoofd ;-)) We horen nog van elkaar! Kelly, immer gemotiveerd, altijd goedlachs en van de 'keep-it-simple' mentaliteit, van dit soort zijn er te weinig op kliniek. Bedankt voor de leuke sfeer en aangename babbels. Geniet samen met Jan van je gezinnetje. Eva en Daphné, bedankt voor alle goede zorgen voor de hospi-patiënten. Maarten Haspeslagh, tot binnenkort eens aan de waterkant, zeker? Succes met je onderzoek. Mireia, veel succes vrijdag! Thomas, Julie, Diego, (the ever smiling) Ilaria en Anna, succes met jullie residency-programma en het examen! Ik duim alvast, good luck! Anja, veel plezier met de anesthesieën. Bedankt aan alle interns van de laatste 6 jaar voor jullie tomeloze inzet en de toffe sfeer tijdens de nachtdiensten: Evi, Babette, Evelyn, Jan, Kimry, Veronika, Evelien, Anaïs, Emmanuelle, Mathias, Sjoerd, Ellen, Joana, Elke en Stephanie: merci! Lore en Cindy De Baere, het was altijd fijn babbelen over de middag, veel geluk in de toekomst en Lore, succes met de verbouwingen en je onderzoek. Johan, bedankt voor alle administratieve en financiële verrichtingen. Aan de dames van de sterilistatie: bedankt voor al het werk dat jullie verzetten. Voor alle textielgerichte vragen weet ik je te vinden, Caroline. Enuh, blijven wandelen eh! Valerie, je passie en liefde voor dieren siert je. Bedankt voor de leuke tijd en alle hulp die ik van je kreeg. Cindy, bedankt voor alles. Laat op tijd en stond maar van jullie horen! Heidi, ongelooflijk hoe je de gebruiksaanwijzingen van ieder van ons op de vakgroep zo perfect weet te hanteren. Chapeau! Bedankt voor de hulp bij 'pagina 26' en om van dit boekje

Dankwoord

een esthetisch verantwoord geheel te helpen maken. Kortom: merci voor alles!! Veerle, bedankt voor de fijne samenwerking en je hulp als ik weer eens met vragen aan je bureau stond. Nadine, Didier, Wilfried, Freddy, Ingbert en Bart, merci om de stallen vlekkeloos te onderhouden. Didier, Jan, Nico en Remi, jullie wisten vaak al hoe laat het was als ik in de smidse kwam opdagen. Bedankt voor alle hulp bij de thomasspalken. Annemie en Hubert, bedankt om het geheel steeds prachtig te onderhouden. Lesley, en haar ISS-collega's, merci om steeds met een glimlach mijn bureau en de administratieve blok proper te maken. Maarten en Eline: dat jullie hechte samenwerking mag resulteren in een succesvol doctoraatswerk. Anyhow, the 24th of December, that day I will always remember...

Graag wil ik ook alle medewerkers van de dienst Inwendige Ziekten, Medische Beeldvorming, Anatomie, Kleine Huisdieren, Verloskunde, Pathologie en Parasitologie danken voor de aangename tijd op en/of naast de werkvloer!

Niet alleen mijn collega's, maar ook de mensen uit mijn persoonlijke omgeving zou ik willen vermelden bij het beëindigen van dit doctoraat. Hierbij wil ik mijn vrienden bedanken voor alle aanmoedigingen en alle fijne momenten van ontspanning naast het werk door de jaren heen! Mama en papa, in alles wat ik ondernam, hebben jullie hebben me steeds door dik en dun gesteund. Bedankt hiervoor en voor alle mogelijkheden die jullie mij tot nu toe in mijn leven gegeven hebben!

Tot slot, Mathieu. Je slaagt er telkens in om in een mum van tijd een lach op mijn gezicht te toveren, ook als het eens wat minder gaat. Bedankt om er steeds voor mij te zijn als het nodig is en gewoonweg om mijn complementaire wederhelft te zijn. Mijn leven met jou maakt me gelukkig. Ik kijk uit naar alle toekomstige avonturen die ons te wachten staan!

Caroline

*'Life is like riding a bicycle.
To keep your balance, you must keep moving.'*

Albert Einstein

