# Comparison of palmar digital neurectomy by the guillotine technique and palmar digital neurotomy associated with end-to-side neurorrhaphy in mares

*Comparação entre neurectomia por técnica de guilhotina e neurotomia associada à neurorrafia em alça de balde em nervo digital palmar de éguas*

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## Abstract

Five mares were submitted to palmar digital neurectomy by the guillotine technique and palmar digital neurotomy followed by end-to-side neurorrhaphy (right and left thoracic limbs, respectively). Mares were checked for local pain sensation using hoof tester and submitted to lameness workup at 15-day intervals. No evidence of painful neuroma formation was detected. Palmar digital nerve (PDN) stump segments were collected within 60 days of surgery. Mean left and right limb PDN stump thickness corresponded to 5.96 mm and 7.16 mm, respectively. Schwann cells prevailed over connective healing tissue in all PDN stumps studied. Well-formed nerve-like structures with better organized nervous tissue and predominance of parallel nerve fiber orientation were documented in left limb PDN stumps. End-to-side neurorrhaphy tended to promote tissue organization, potentially reducing the chances of neuroma formation.

**Keywords:** Neuroma. Peripheral nerve. Equine. Surgery.

## Resumo

O nervo digital palmar (NDP) lateral do membro torácico direito (MTD) de cinco equinos fêmeas foi submetido à neurectomia pela técnica da guilhotina, e o do membro torácico esquerdo (MTE) à neurotomia e neurorrafia término-lateral. Os animais foram avaliados a cada 15 dias quanto ao teste de sensibilidade cutânea com pressão local com pinça de casco e de claudicação, não sendo notados sinais clínicos de neuroma doloroso. Aos 60 dias pós-cirurgia coletou-se segmentos dos cotos proximais dos NDPs. Os dos MTDs apresentavam em média, a espessura de 7,16 mm e aos dos MTEs de 5,96 mm. Nos cotos proximais dos nervos dos membros direito e esquerdo notou-se predominância de células de Schwann à grande quantidade de tecido conjuntivo cicatricial. Os do MTEs apresentavam estruturas de nervo típico, bem constituídas, com maior organização do tecido nervoso e predomínio de fibras nervosas orientadas paralelamente. A neurorrafia termino-lateral apresentou tendência a ocasionar maior organização entre as estruturas analisadas, o que lhe conferiu menor potencial em desenvolver neuromas dolorosos.

**Palavras-chave:** Neuroma. Nervo periférico. Equinos. Cirurgia.

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## Introduction

Navicular disease is one of the most common causes of chronic lameness in show jumpers and race horses and constitutes one of the major indications for palmar digital neurectomy. The procedure is also indicated in conditions such as distal phalanx and navicular bone fractures, ossification of the collateral cartilages, pedal osteitis or any other condition requiring desensitization of distal limb structures (PEIXOTO; SOUZA, 2004; ESCOBAR et al., 2008). Neuroma formation, rupture of the deep digital flexor tendon, loss of the hoof capsule, palmar digital nerve regeneration and incomplete desensitization of the heel area are some of the complications of palmar digital neurectomy (STASHAK, 1994; DABAREINER et al., 1997).

Neuromas reflect disorganized axon regeneration attempts and constitute a common change developing at injured nerve endings. Neuroma formation is associated with Schwann cell proliferation in several directions and branching into perineural fibrous connective tissue formed at surgical sites as part of the normal healing process. Neuromas are painful to pressure and tension, and/or when submitted to hypoxia (HAUGLAND et al., 1992; VITERBO et al., 1992; MARTINS et al., 2002). Pain responses are thought to result from hypersensitivity and spontaneous activity of regenerating axons (CUMMINGS et al., 1988). Painful neuromas are career-ending lesions in athletic horses; the incidence of neuroma formation has not been well documented, but is thought to be 20-34% on average (TURNER et al., 1995).

Different surgical techniques have been proposed to prevent or reduce disorganized axonal growth, such as radioactive cotton ligature (GORMAN et al., 1962), cryoneurectomy (TATE; EVANS, 1980), $CO_2$ laser neurectomy (HAUGLAND et al., 1992), epineural capping (DABAREINER et al., 1997), stripping (FALEIROS et al., 2008), silicon tube suture (FERREIRA et al., 2002; DELISTOIANOV et al., 2006), application of sodium hyaluronate (MURRAY et al., 1994) or N-butyl cyanoacrylate (TURNER et al., 1995), and administration of neurotoxic substances (CUMMINGS et al., 1988).

Viterbo (1992) has reintroduced the end-to-side neurorrhaphy concept, supporting the efficacy of the technique. End-to-side neurorrhaphy has also been employed for treatment of distal limb neuromas (BERTELLI et al., 1996; NOAH et al., 1997; ROSSETO et al., 2001) and prevention and treatment of experimentally induced painful neuromas in mice (Al-QATTAN, 2000).

This study set out to compare the effects of palmar digital neurotomy associated with end-to-side neurorrhaphy and palmar digital neurectomy using the guillotine technique in prevention of neuroma formation in horses.

## Material and Methods

This project had the approval of the Animal Experimentation Ethics Committee (protocol No. 12871/2008/UNIPAR). Five mixed-breed mares aged between three and five years were used. Mares were kept in individual paddocks during a 30-day adaptation period, dewormed with moxidectin (0.4 mg/kg), trimmed and submitted to lameness workup at the walk and trot. Feeding regimen consisted of hay, commercial horse feed, salt and grounded corn; water was offered *ad libitum*.

Mares were submitted to food and water withdrawal (24 h and 8 h, respectively) and sedated with intramuscular xylazine hydrochloride and acepromazine maleate (1.0 mg, kg$^{-1}$, and 0.05 mg, kg$^{-1}$ respectively). Induction was performed with intravenous infusion of glyceryl guaiacolate in 5% glucose solution and ketamine (1.0 g). Anesthesia was

maintained with halothane in 100% oxygen (10 ml.kg$^{-1}$.min$^{-1}$) in semi-closed circuit system.

The lateral aspect of both thoracic limbs was clipped from the upper limit of the pastern to the coronary band; the skin was prepared with povidone-iodine and 70% alcohol and a tourniquet applied to the midmetacarpal area. A 4.0 cm incision was made through the skin at the posterior third of the lateral aspect of the pastern and the subcutaneous tissues separated to expose the lateral palmar digital nerve. On the right thoracic limb (RTL), the lateral palmar digital nerve was isolated and a 2.0 cm segment resected using the guillotine technique (TURNER; MCILLWRAITH, 2002). On the left thoracic limb (LTL), the corresponding nerve was transected and the proximal end folded back on itself to form a loop; the epineurium was then incised under a surgical microscope and the nerve stump introduced into the incision and secured with interrupted 9-0 polyglactin 910 sutures.

The skin was closed with 1-0 nylon suture and surgical wounds covered with povidone-iodine soaked gauze sponges. Operated limbs were placed in cotton/elastic tape bandages extending from the coronary band to the metacarpophalangeal joint. Bandages were changed every two days for a 10-day period and sutures removed within 10 days of surgery. Phenilbutazone (2.2 mg.kg$^{-1}$) was given intravenously once daily for three days following surgery.

Over the course of the 60-day postoperative follow-up period, mares were checked for local pain sensation every 15 days by means of hoof tester application to the operated area, with different degrees of pressure. Lameness workup was also performed; lameness was graded according to a 0-to-5 scale (BAXTER; STASHAK, 2011), as follows: 0 – not lame; 1 – inconsistent lameness, difficult to recognize regardless of circumstances; 2 – lameness difficult to recognize when walking or trotting in a straight line, apparent under stress circumstances; 3 – consistent lameness, obvious at the trot; 4 – obvious lameness, head

movements, sudden lifting of weight-bearing limb, shortened stride; and 5 – minimum weight bearing, weight bearing avoided when moving or at rest. Local pain sensation was graded according to a 0-to-3 scale (FALEIROS et al., 2008), as follows: 0 – absent, 1 – reaction to strong pressure; 2 – normal (expected) reaction to moderate pressure; 3 – exaggerated reaction to mild pressure.

On postoperative day 60, mares were again submitted to aforementioned anesthetic and preoperative skin preparation protocols for gross inspection of adjacent tissues and investigation of new tissue formation and adhesions. Two (2)-cm segments were collected from the proximal end of the guillotined lateral palmar digital nerve and the neurorrhaphy site (right and left thoracic limbs respectively) in block, after dissection of the superficial tissue. Surgical wounds were treated according to previous description.

Proximal nerve stump specimen thickness was measured using caliper. Specimens were then fixed in Bouin's solution, dehydrated in increasing concentrations of ethanol, diafanized in xylol, embedded into paraffin blocks and sectioned in microtome for slide preparation. Slides were stained with hematoxylin-eosin (HE) and Masson's trichrome (MT) and submitted to light microscopy for investigation of neuroma formation and presence of inflammatory cells, quantification of nerve fibers and determination of nerve fiber orientation, and Schwann cell counting. Quantification of collagen fibers was also performed; collagen fibers were quantified according to a 0-to-4 scale, as follows: 0 – absent; 1 – small amounts; 2 – moderate amounts; 3 – large amounts; 4 – exaggerated amounts.

## Results and Discussion

Surgical interventions and anesthetic recovery were uneventful in this study. Mild to moderate postoperative edema developed at the pastern and fetlock level in both operated limbs, which subsided within 20 days of surgery. Postoperative edema

following palmar digital neurectomy has been reported elsewhere (MURRAY et al., 1994; FALEIROS et al., 2008) and is thought to be a common occurrence in this type of surgery.

Partial wound dehiscence (two skin sutures) was observed in one mare (20%) in this sample within one week of surgery. Surgical wound healing was otherwise uneventful, with absence of oozing or infection in all cases (100%).

Assessment of local pain sensation in response to hoof tester application revealed grade 3 (exaggerated) reactions in both limbs in four (80%) mares and grade 2 (normal) reactions in one (20%), within 15 days of surgery; within 30 days, one mare (20%) still showed grade 3 reaction, while grade 1 and grade 2 reactions were manifested by 1 (20%) and 3 (60%) mares, respectively. Within 45 and 60 days of surgery, three mares (60%) had grade 1 and two mares (40%) grade 2 (normal) reactions (Table 1). Exaggerated pain responses to hoof tester application to the operated area have been reported by Delistoianov et al. (2006) in a comparative study of two different palmar digital neurorrhaphy techniques in horses, and by Dabareiner et al. (1997) during the first 30 postoperative days in a trial comparing the guillotine technique with different $CO_2$ laser neurectomy techniques.

Increased local sensitivity was also documented by Turner et al. (1995) in horses submitted to three different neurectomy techniques. In this study, increased ability to react to local pressure in the first days of the postoperative period may have reflected edema and inflammation in response to surgical trauma, with increased sensitivity of nerve endings surrounding the desensitized area (FALEIROS et al., 2008).

All five mares (100%) were lame within 15 days of surgery; lameness was graded 2 and 1 in two (40%) and three (60%) mares, respectively. Within days 30, grade 1 lameness persisted in four mares (80%), while one (20%) was no longer lame. Within 45 days, grade 1 lameness still persisted in two (40%) mares, with lameness remission in the remaining ones. Only one mare (20%) remained lame within 60 days of surgery. However, despite persistence of inconsistent (grade 1) lameness in this mare, reaction to hoof tester application was normal (grade 2); therefore, the possibility of neuroma formation was dismissed (Table 1).

Table 1 – Degree of lameness (L) and local pain sensation (S) in five mares submitted to palmar digital neurectomy by the guillotine technique and palmar digital neurotomy associated with end-to-side neurorrhaphy (right and left thoracic limbs, respectively), at different postoperative time points – Umuarama, PR – 2009

| Postoperative time point | 15 days L S | 30 days L S | 45 days L S | 60 days L S |
|---|---|---|---|---|
| Mare 1 | 1 3 | 1 2 | 0 1 | 0 1 |
| Mare 2 | 2* 3* | 1* 3* | 1* 2 | 0 2 |
| Mare 3 | 1 2 | 0 1 | 0 1 | 0 1 |
| Mare 4 | 2 3 | 1** 2 | 1** 1 | 0 1 |
| Mare 5 | 1 3 | 1** 2 | 1** 2 | 1** 2 |

* predominantly left thoracic limb; **predominantly right thoracic limb. Lameness: 0 – not lame; 1- inconsistent lameness; 2 – lameness difficult to recognize at the walk or trot, apparent under stress; 3 – obvious consistent lameness at the walk and trot; 4 – obvious lameness; 5 – non-weight bearing when moving or at rest. Sensitivity: 0 – absent, 1 – reaction to strong stimulus; 2 – normal reaction to moderate stimulus; 3 – exaggerated reaction to normal stimulus

Different palmar digital neurectomy techniques have been evaluated in horses (HAUGLAND et al., 1992; MURRAY et al., 1994; DABAREINER et al., 1997; FALEIROS et al., 2008). As in this study, no clinical evidences of painful neuroma formation were documented. Postoperative lameness workup in this study suggests that lameness tends to resolve over the course of the postoperative period following palmar digital neurectomy.

Mild to moderate or moderate to severe degrees of adhesion (LTL and RTL, respectively) were documented upon gross inspection of operated sites in this study; the higher the degree of adhesion, the more difficult the dissection and isolation of the palmar digital nerve. Adhesion formation following palmar digital neurectomy or neurorrhaphy has been reported elsewhere (DELISTOIANOV et al., 2006). Scar tissue formation was noted in all proximal palmar digital nerve stumps in this sample. Mean proximal palmar digital nerve stump thickness corresponded to 5.96 mm and 7.16 mm (LTL and RTL, respectively), suggesting more pronounced scar tissue formation following neurectomy compared to neurorrhaphy. Scar tissue formation at nerve stumps may indicate neuroma development (i.e., non-neoplastic thickening at transected nerve stumps) and

is to be expected following this type of surgical procedure (ESCOBAR et al., 2008).

Histological analysis suggested new nervous tissue formation in all neurorrhaphy and in 60% of neurectomy stumps in this sample. Newly formed nervous structures seen on histology were either devoid of or wrapped in thin, discrete perineurium. Thin parallel, concentric nerve fibers that tended to form a bundle, Schwann cells and evidences of axons and myelin sheath were also observed. Nerve-like structures wrapped in perineurium and containing axons and clearly distinguishable myelin sheath, as well as smaller numbers of Schwann cells, were seen in larger numbers in two (20%) out of five neurorrhaphy stumps. Nerve fibers and Schwann cells in parallel orientation prevailed in all (100%) neurorrhaphy stumps. Nerve-like structures were less organized and widely intermingled with fibrous tissue in two (40%) out of five neurectomy stumps. With the exception of one mare (20%), larger amounts of collagen fibers were documented in neurectomy compared to neurorrhaphy stump specimens of the same animal. Few inflammatory infiltrates and blood vessels were seen in all (100%) nerve stumps analyzed, regardless of the technique employed (Table 2; Figure 1).

Table 2 – Histological findings in palmar digital nerve stump specimens of five mares submitted to neurectomy by the guillotine technique and neurotomy associated with end-to-side neurorrhaphy (right and left thoracic limbs, respectively) – Umuarama, PR – 2009

| Animal No. | I | II | III | IV | V |
|---|---|---|---|---|---|
| Histological features | RTL LTL | RTL LTL | RTL LTL | RTL LTL | RTL LTL |
| Nerve-like structure | Abs. Abs. | Abs. Pres. | Abs. Abs. | Abs. Pres. | Abs. Abs. |
| New nervous tissue formation | Abs. Pres. | Aus. Pres. | Pres. Pres. | Pres. Pres. | Pres. Pres. |
| Orientation of nerve fibers (predominant) | Diff. Paral. | Paral. Paral. | Paral. Paral. | Diff. Paral. | Paral. Paral. |
| Collagen fibers (intensity)* | 4 3 | 4 2 | 3 4 | 4 2 | 2 1 |
| Inflammatory infiltrate and blood vessels | Few Few | Few Few | Few Few | Few Few | Few Few |

*Intensity: 1 = minimum, 2 = low, 3 = moderate, 4 = high, 5 = very high. RTL = right thoracic limb. LTL = left thoracic limb. Abs.: Absent; Pres. Present; Diff.: Diffuse; Paral: Parallel
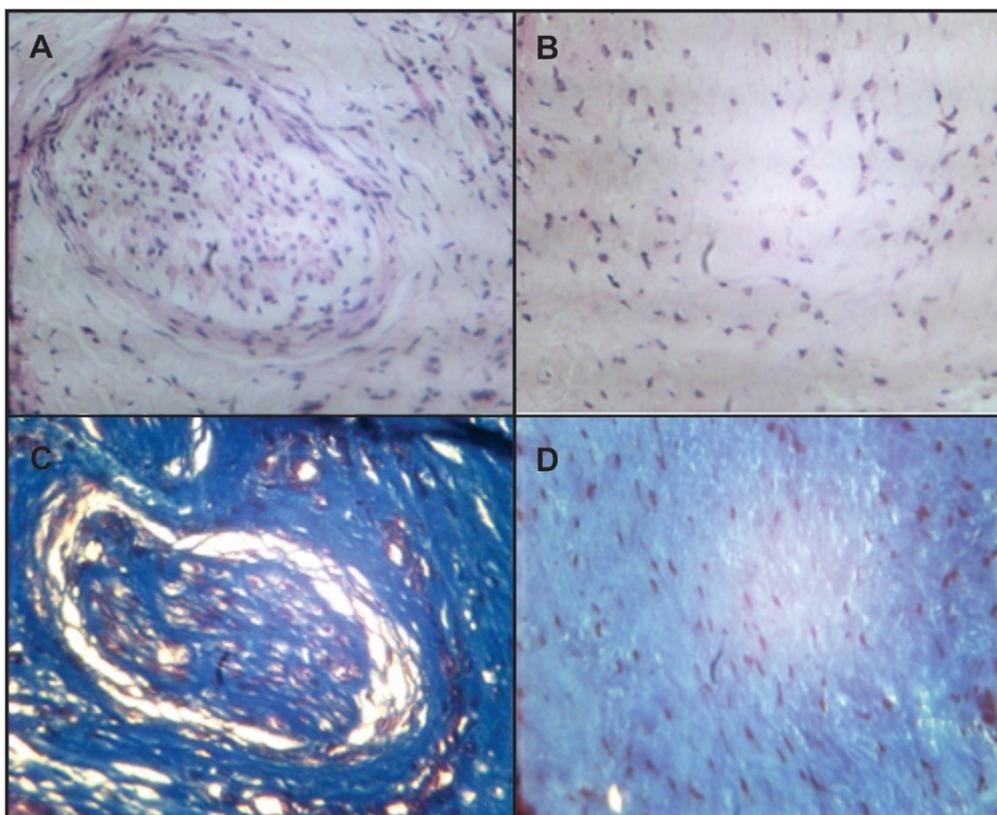
Figure 1 – Photomicrograph (postoperative day 60) of palmar digital nerve stump specimens of mares submitted to neurotomy associated with end-to-side loop neurorrhaphy and palmar digital neurectomy using the guillotine technique. (A) Left thoracic limb (LTL); nerve-like structure wrapped in perineurium, containing axons and clearly distinguishable myelin sheath – HE. (B) Right thoracic limb (RTL); disorganized Schwann cells in diffuse and parallel orientation, intermingled with scar tissue – HE. (C) LTL; nerve-like structure – MT. (D) RTL; disorganized Schwann cells and large amounts of collagen fibers – MT. 40 X magnification

On histology, nerve bundles appeared dispersed in abundant collagen matrix and several cord-like structures comprised of Schwann cells. Fibrovascular tissue proliferation around proximal nerve stumps with small numbers of nerve fibers and Schwann cells, as well as minimum signs of chronic inflammation, have been reported upon examination of the palmar digital nerve of horses submitted to three different neurectomy techniques, and are thought to be consistent with the definition of neuroma (TURNER et al., 1995).

Marked scar tissue formation, which actually prevailed over nerve tissue formation in this study, is thought to reflect the characteristic tendency of horses to manifest pronounced healing process and disorganized peripheral nerve regeneration, with prominent fibrous connective tissue proliferation (DELISTOIANOV et al., 2006).

In a study by Al-Qattan (2000), classical neuromas (i.e., disorganized nervous tissue and soft tissue infiltration) developed at the proximal stump of transected peripheral nerves, in contrast to non-classical neuromas (i.e., better organized nervous tissue) in nerve stumps submitted to end-to-side neurorrhaphy. Based on histological criteria given by Al-Qattan (2000), digital nerves submitted to neurectomy by the guillotine technique developed classical neuromas, while non-classical neuromas were documented in nerves submitted to neurorrhaphy in this trial.

## Conclusions

Mares submitted to lateral palmar digital neurotomy associated with end-to-side loop neurorrhaphy and lateral palmar digital neurectomy developed non-painful neuromas over the course of the 60-day experimental period. Histological findings suggest peripheral nerves submitted neurotomy and end-to-side loop neurorrhaphy are less prone to painful neuroma formation.

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