

DOI: 10.1111/vsu.13548

ORIGINAL ARTICLE - RESEARCH

WILEY

Ex vivo study of vagal branches at risk for iatrogenic injury during laryngoplasty in horses

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Funding information

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Abstract

Objective: To localize vagal branches within the surgical field of laryngoplasty and identify potentially hazardous surgical steps.

Study design: Observational cadaveric study.

Sample population: Five equine head–neck specimens and four entire equine cadavers.

Methods: Dissection of the pharyngeal region from a surgical perspective. Neuronal structures were considered at risk if touched or if the distance to instruments was less than 5 mm.

Results: The branches of the pharyngeal plexus (PP) supplying the cricopharyngeal muscle (PPcr), the thyropharyngeal muscle (PPth), and the esophagus (PPes) were identified in the surgical field in nine of nine, five of nine, and one of nine specimens, respectively. The internal branch of the cranial laryngeal nerve (ibCLN) was identified within the carotid sheath in six of nine specimens. The external branch of the cranial laryngeal nerve (ebCLN) was identified close to the septum of the caudal constrictors in nine of nine specimens.

The blade of the tissue retractor compressed the ibCLN in six of six, the ebCLN in four of six, the PPcr in six of six, the PPth in two of three, and the PPes in two of two specimens in which the respective nerves were identified after further dissection. Surgical exploration of the dorsolateral aspect of the pharynx and the incision of the septum of the caudal constrictors harmed the ebCLN in nine of nine, PPcr in seven of nine, and PPth in four of eight specimens.

Conclusion: Several vagal branches were located in the surgical field and must be considered at risk because of their location.

Clinical significance: Use of the tissue retractor, dissection over the pharynx, and dissection of the septum of the caudal constrictors involve a risk to damage vagal branches.

1 | INTRODUCTION

Prosthetic laryngoplasty is commonly used to treat laryngeal hemiplegia in athletic horses, although serious postoperative complications such as dysphagia¹⁻⁵ and esophageal reflux⁶⁻⁹ may occur. Postoperative tracheal contamination and subsequent coughing, nasal discharge, and potentially fatal pulmonary diseases may

result from mechanical impact impairing airway protection during swallowing (eg, overabduction of the arytenoid cartilage, aggressive removal of vocal cord and ventricle) or functional disturbances (swallowing difficulty or dysphagia).^{2,3,9-14} Coughing and nasal reflux after laryngoplasty have been reported, with a prevalence of up to 57%^{3,10} and 43%,^{4,8,10} respectively. Tracheal contamination and coughing often occur shortly after surgery, and removal of the prosthesis often immediately resolves the clinical signs.^{1,3,4}

In some horses, however, the clinical signs of dysphagia and esophageal reflux have been reported to occur for days to weeks^{1,13,15,16} or for up to 1 year postoperatively¹⁴ and to persist for several months after laryngoplasty.^{7-9,16} In some horses, clinical signs emerged irrespective of an excessive arytenoid abduction^{8,16,17} and did not resolve after removal of the prosthesis.^{1,5,9,12,13,15,18} Moreover, a direct association between the surgical procedure and swallowing disturbances has been suspected after sham laryngoplasty.¹² A specific cause for postoperative dysphagia has not been determined, and the disorder probably results from a multifactorial cause.

Swallowing is a complex neuromuscular process elicited by the cranial nerves and consists of a voluntary oral phase and two reflexive pharyngeal and esophageal phases.¹⁹⁻²¹ The vagus and the glossopharyngeus nerves provide motor innervation to the pharyngeal, soft palate muscles, and cervical esophagus and sensory innervation to the pharvngeal and larvngeal mucosa to a variable extent.¹⁹⁻²² The vagus nerve is considered the key element of the swallowing reflex. The vagus nerve provides efferent fibers to the pharyngeal and laryngeal muscles via the pharyngeal plexus (PP) and the external branch of the cranial laryngeal nerve (ebCLN) and sensory fibers through the internal branch of the cranial laryngeal nerve (ibCLN).¹⁹⁻²¹ The PP consists of two main components, the pharyngeal branches of the vagus and glossopharyngeus nerves on one hand and contributions from the CLN and the cranial cervical ganglion on the other hand.^{20,21} The PP supplying the esophagus (PPes) provides motor innervation to the cervical esophagus and the caudal pharyngeal constrictor muscles.^{21,23} The ebCLN innervates the cricothyroid muscle, while the ibCLN provides sensory innervation to the mucosa of the laryngeal vestibule, ventral and dorsal pharynx, caudal portion of the soft palate, and cervical esophagus.^{10,21,23,24} Surgical trauma to the caudal pharyngeal constrictor muscles, the cervical esophagus (including the vestibulum esophagi of the pharynx), and the branches of the vagus nerve (the CLN, its internal and external branches [ibCLN and ebCLN, respectively], and the PPes) has been suspected to result in pharyngoesophageal

dysfunction after laryngoplasty,^{2,9-13,25} although no putative critical steps have been identified.

The objective of this study was to identify the branches of the vagus nerve (CLN and PP) within the surgical field of laryngoplasty and the critical steps during the laryngoplasty potentially leading to iatrogenic injuries of these branches.

2 | MATERIALS AND METHODS

2.1 | Animals and specimens

Nine mixed breed adult equine cadavers of average size and similar weight (age range: 8-22 years; five males and four females) and without any known pathologic changes of the pharynx were used. The client-owned horses had been euthanized for clinical reasons unrelated to this study and exsanguinated by transecting both jugular veins. Informed owner consent for unspecific research studies was obtained.

Four cadavers were left entire (n = 4), while headneck specimens (n = 5) were obtained by severing the neck of five cadavers between the fifth and sixth cervical vertebra immediately after euthanasia. The trachea of the head-neck specimens was pulled caudally and fixed to reverse the rostral displacement of the larynx and surrounding neurovascular structures.

2.2 | Laryngoplasty

A left-sided laryngoplasty was performed by a European College of Veterinary Surgery diplomate surgeon experienced in upper airway surgery (G.B.). The head was hyperextended, and the plane between the linguofacial vein and the omohyoid muscle was dissected (step 1). The blade tip of a 21-cm hand-held Kocher-Langenbeck tissue retractor (Gribi AG, Belp, Switzerland) was stained with black ink (Edding AG, Ahrensburg, Germany) and placed under the linguofacial vein to access the ventral cervical space from step 2 to step 7. The dorsolateral aspect of the larynx and the septum of the caudal pharyngeal constrictor muscles were dissected both sharply and bluntly (step 2). The spine of the muscular process was grasped by a 25-cm sharp Schroeder tenaculum forceps (Diener GmbH, Tuttlingen, Germany) to elevate and slightly rotate the arytenoid cartilage outward (step 3). A custom-made prosthesis was prepared by fixing a 8 USP pseudomonofilament polycaprolactam suture (Supramid; WDT Wirtschaftsgenossenschaft Deutscher Tierärzte eG, Garbsen, Germany) at the end of a curved 14-gauge hypodermic needle (Sterican; B Braun Medical SA,

Sempach, Switzerland) and placed through the muscular process of the arytenoid cartilage (step 4). The hypodermic needle was removed, and both ends of the prosthesis were pulled caudally underneath the cricopharyngeal muscle by using a hemostat (Provet AG, Lyssach, Switzerland; step 5). The caudolateral aspect of the cricoid cartilage was grasped with a 25-cm sharp Schroeder tenaculum forceps (Diener GmbH) and rotated outward (step 6). A 20-cm right-hand sharp Deschamps needle Medizintechnik für Tierärzte (Eickemeyer AG. Appenzell, Switzerland) was used to place one end of the prosthesis through the cricoid cartilage (step 7). Finally, the prosthesis was tied (step 8).



The paths of the branches of the vagus nerve in the surgical field were carefully observed during all eight steps. In six specimens, the parotid, pharyngeal, and laryngeal regions were subsequently dissected further, and the vagal branches were tracked to both their target muscle and their origin to confirm their intraoperative identification. Findings were validated with the available literature.^{21-23,26} Neuronal structures were considered at risk during laryngoplasty when they were affected or were closer than 5 mm to the instruments and sutures. One specimen was subsequently fixed in a 4% buffered solution (Grogg Chemie. formaldehvde Stettlen. Switzerland) for illustrative purposes.

3 | RESULTS

3.1 | The path of the branches of the vagus nerve in the surgical field

The surgical field of laryngoplasty corresponded with the cranial portion of the left ventral cervical space (Figures 1-3). The medial surface of the surgical field was consistent with the visceral layer of the deep cervical fascia covering the caudal pharyngeal constrictor muscles and the esophagus. The carotid sheath (Figures 1A and 2B,C) and the medial compartment of the guttural pouch bordered the surgical field dorsally in nine of nine and four of nine specimens, respectively. A thick layer of

FIGURE 1 Neuroanatomy of the laryngoplasty surgical field. Left ventral (A) and ventrolateral (B,C) views of the laryngoplasty surgical field of two fresh (A,B) and one formaldehyde-fixed (C) specimens. A, The internal branch of the cranial laryngeal nerve (ibCLN) and various branches of the pharyngeal plexus (PP) crossed the carotid sheath (CS) within the buccopharyngeal fascia. Lymphatic vessels (LV) had a similar course and were difficult to distinguish from small nerve branches without further dissection. The ibCLN coursed shortly along the CS over the thyropharyngeal muscle (TPM) before leaving the surgical field. The external branch of the cranial laryngeal nerve (ebCLN) descended within the septum between the TPM and the cricopharyngeal muscle (CPM) toward the cricothyroid muscle. The borders of the tissue retractor (asterisk) are outlined with dashed lines. B, Additional dissection of the fascia exposed the branch of the PP innervating the CPM (PPcr) and the ebCLN. The thick adipose tissue ventral to the septum is marked with thick dashed lines. The blade of the tissue retractor is indicated with an asterisk. C, Close view on the septum between TPM and CPM after dissection of the fascia. Two branches of the pharyngeal plexus descended over the CPM and the septum before innervating the TPM (PPth)



FIGURE 2 The pressure zone of the tissue retractor in the laryngoplasty surgical field. A, Dorsolateral view of the pharyngeal and laryngeal regions. The skin and the subcutaneous tissue overlying the surgical field were dissected. The position of the blade of the tissue retractor (asterisk) in the surgical field corresponded with the course of the internal branch of the cranial laryngeal nerve (ibCLN). B,C, Ventral views of the surgical field after placement of a tissue retractor with the blade (asterisk) stained with black ink. The borders of the tissue retractor are outlined with dashed lines. The pressure zone of the tissue retractor (identifiable as black spots due to applied ink) corresponded to the location of the dorsal aspect of the buccopharyngeal fascia, the carotid sheath (CS), the ibCLN, the external branch of the cranial laryngeal nerve (ebCLN), and the branches of the pharyngeal plexus (PP). AC, ansa cervicalis; CPM, cricopharyngeal muscle; ebCLN, external branch of the cranial laryngeal nerve; LV, lymphatic vessels; PPcr, branch of the PP innervating the CPM; TPM, thyropharyngeal muscle

adipose and connective tissues covered the caudal pharyngeal constrictor muscles and formed the ventral and caudal border of the surgical field in all the specimens (Figures 1B, 2B, and 3B). The trunk of the vagus nerve and the origin of the PP and CLN were outside the surgical field in all the specimens.

At least one of the terminal branches of the PP was visualized within the visceral layer of the deep cervical fascia in the surgical field of all specimens. The branches coursed with a highly variable pattern along the carotid sheath before descending and eventually piercing the caudal pharyngeal constrictor muscles and the cervical esophagus. The branches supplying the thyropharyngeal muscle (PPth) and the cricopharyngeal muscle (PPcr) were identified within the surgical field in five of nine and nine of nine specimens, respectively. Additional dissection allowed the detection of the PPth in an additional three specimens. The PPcr perforated the lateral and the caudal aspect of the cricopharyngeal muscle in seven of nine and two of nine specimens, respectively. The esophageal branch of the PP (PPes) was identified within the surgical field in only one specimen. Additional dissection allowed the detection of the PPes in an additional specimen. The PPes coursed along the ventrolateral aspect of the cervical esophagus, very close to the PPcr (Figure 3A,C), and was almost completely covered by adipose and connective tissue (Figure 3).

The thick ibCLN was visualized in the dorsal aspect of the surgical field in six of nine specimens. Futher dissection allowed the detection of the ibCLN in an additional specimen. The ibCLN coursed rostrally within the visceral layer of the deep cervical fascia along the carotid sheath (Figures 1 and 2C). The ibCLN crossed underneath the carotid sheath from dorsomedial to ventrolateral and left the surgical field cranially after a short course over the dorsal aspect of the thyropharyngeal muscle.

The thin ebCLN was identified within the visceral layer of the deep cervical fascia in the surgical field of nine of nine specimens. The ebCLN descended ventrally close to the septum of the caudal pharyngeal constrictor muscles toward the cricothyroid muscle (Figures 1A,B and 2B). The ebCLN additionally supplied the thyropharyngeal muscle in one specimen.

3.2 | Iatrogenic injuries during laryngoplasty

As confirmed by black staining (Edding AG), the blade of the tissue retractor (Gribi AG) compressed the carotid sheath, the visceral layer of the deep cervical fascia, and the ibCLN (Figure 2A,C) from step 2 to step 8 in all six



FIGURE 3 The esophageal branch of the pharyngeal plexus (PPes) in the surgical field of laryngoplasty. A, Ventrolateral view of the caudodorsal aspect of the surgical field. The PPes descended close to the cricopharyngeal branches of the pharyngeal plexus (PPcr) to innervate the lateral aspect of the cervical esophagus (ES). The PPcr then pierced the cricopharyngeal muscle (CPM). B, Left caudolateral view of a dissected pharynx after laryngoplasty (ex situ). The knotted suture (triangle) lay in the thick connective and adipose tissue (dashed lines), close to the course of the PPes. The external branch of the cranial laryngeal nerve (ebCLN) coursed along the septum between the CPM and the thyropharyngeal muscle. C, Lateral view of the caudoventral aspect of the laryngoplasty field after additional dissection in a formaldehyde fixed specimen. The PPcr and the main trunk of the PPes descended close to each other. The PPes then descended over the lateral aspect of the cervical ES giving off several secondary branches (asterisks), dorsal to the cranial thyroid veins (CTV), cranial thyroid artery (CTA), and recurrent laryngeal nerve (RLN)

specimens in which the ibCLN was identified in the surgical field. Additional dissection revealed a correspondence between the pressure zone of the tissue retractor (Gribi AG) and the path of the PPcr in six of six, the ebCLN in four of six, the PPth in two of three, and the PPes in two of two specimens in which the respective nerves were identified (Figure 2). Blunt dissection of the dorsolateral aspect of the larynx (step 2) exposed the PPcr in seven of nine, the ebCLN in nine of nine, and the PPth in four of eight specimens in which the respective nerves were identified (Figures 1C, 2B,C and 3A). While the ventral aspect of the septum of the caudal pharyngeal constrictor muscles was incised (step 2), the ebCLN had to be carefully dissected and retracted in six of nine specimens to prevent its transection (Figure 1). The caudodorsal border of the cricoid cartilage was bluntly prepared and freed from the loose connective tissue. The Deschamps needle was inserted through the caudal

aspect of the cricoid cartilage (step 7) approximately 5 mm lateroventral to the path of the PPes. The tied prosthesis (step 8) compressed the adipose tissue close to the PPes and entrapped the ebCLN in one specimen.

4 | DISCUSSION

Intraoperative neural damage has been suspected to cause dysphagia after laryngoplasty in horses.^{2,9-13} A better understanding of the neuroanatomy of the pharyngeal and laryngeal regions can help to prevent intraoperative injuries to relevant nerves. This cadaveric study reports the course of the branches of the PP and of the CLN (both originating partially or completely from the vagus nerve) in the surgical field for prosthetic laryngoplasty and identifies the critical steps of the surgical procedure that may result in neuronal injury.

4.1 | The path of the branches of the vagus nerve in the surgical field

The neuroanatomy of the vagal branches had considerable individual variability, and it was quite challenging to detect these branches in the surgical field. Precise anatomical and physiological knowledge of the PP including number, variation, anatomical relationships, and target structures of its branches is lacking in horses, and it has only recently been expanded in man.²⁷ For example, only the PPes has previously been named separately in horses.^{20,21} In this study, the origin of the PP lay too deeply to be visualized in the surgical field of any specimen. The PP branches displayed a highly variable pattern and were difficult to differentiate from the surrounding fasciae. Only the PPcr (Figures 1B, 2C, and 3A,C) could be visualized in the surgical field of all the specimens, while additional dissection was usually required to visualize the PPth and the PPes (Figure 3), which even then eluded scrutiny in some specimens. Therefore, the PPth and PPes can hardly be detected intraoperatively, and surgeons should rely on their knowledge of the course of the nerves rather than on direct visualization.

The variability of the neuroanatomy of the CLN has been described in more detail in horses. In agreement with our results, the CLN mainly arises from the vagus nerve and forks at the level of the bifurcation of the common carotid artery into the ibCLN and the ebCLN.^{20,21} Moreover, the ebCLN may arise directly from the vagus nerve or, exceptionally, from its pharyngeal branch^{20,21} or, controversially, from the PPes.²³ In this study, the CLN lay too deeply in the surgical field and could not be identified in any specimen. The thicker ibCLN was easily visualized in the surgical field in more than half of the specimens as it coursed within the fascia along the carotid sheath and over the dorsal aspect of the caudal pharynx (Figures 1 and 2C), without laying directly on the pharynx as it is often shown in anatomy Abundant adipose books. tissue may impair intraoperative direct visualization. However, the carotid sheath should be used as a landmark. The thinner ebCLN was difficult to differentiate from the surrounding fascia but could be visualized in all the specimens very close to the septum of the caudal pharyngeal constrictor muscles (Figures 1A,B and 2B), as reported previously.^{10,17,26} In one specimen, the ebCLN innervated not only the cricothyroid muscle but also the thyropharyngeal muscle, as has recently been described in man.²⁸ To the best of the authors' knowledge, this phenomenon has not been reported in horses, so additional research is required.

4.2 | Iatrogenic neural damage during laryngoplasty

Possible iatrogenic injuries to the branches of the vagus nerve were identified during (1) placement of the tissue retractor (steps 2-7), (2) blunt dissection of the dorsal aspect of the larynx and of the septum of the caudal pharyngeal constrictor muscles (step 2), (3) placement of the prosthesis through the cricoid cartilage (step 7), and (4) tying of the prosthesis (step 8). Insertion and maintenance of the blade of the tissue retractor in the pharyngeal and laryngeal regions and manipulation and blunt dissection at the dorsolateral aspect of the larynx were the surgical steps carrying the highest risk for iatrogenic injuries to the branches of the vagus nerve. The tissue retractor, which was left in place during almost the whole procedure (six of eight steps), compressed the ibCLN, the PPcr, and the PPes in all the specimens, while the PPth and the ebCLN were compressed in six of nine specimens in which the nerves were identified in the surgical field after further dissection (Figure 2). Similarly, the role of the tissue retractor in neurogenic injury to the ibCLN during cervical surgery has recently been highlighted in man.^{29,30} Pressure on nerves usually leads to transient neurapraxia.³¹ However, prolonged or elevated pressure may result in permanent neuronal damage.³² To the best of the authors' knowledge, however, this is the first report of a possible association between the use of a tissue retractor and neuronal damage during equine laryngoplasty; additional studies are required to elucidate the pathogenesis.

The branches of the PP may be damaged during several surgical steps. Exposure of the muscular process through blunt dissection (step 2) and placement of the tissue retractor (steps 2-7) were the most relevant and hazardous steps. Moreover, the course of the PPcr indicates that it may be compressed by the prosthesis if this is tightened above and not below the cricopharyngeal muscle, although this was not investigated in this study.

The ebCLN is at risk during placement of the tissue retractor and during the dissection of the septum of the caudal pharyngeal constrictor muscles (step 2; Figure 1), as previously reported.^{10,17,26} Moreover, the ebCLN may be trapped and compressed by the suture used to close the aponeurosis of the caudal constrictors, although this was not investigated in this study.²

Because of the restricted mobility and the thinness of the ibCLN where it enters the larynx, it has been suggested that the thyroid foramen is the most vulnerable zone for iatrogenic damage of ibCLN in both man²⁹ and horses.¹³ In this study, however, the region of the thyroid foramen lay always too far rostrally to be visualized, and the ibCLN was compressed and stretched by the tissue retractor more dorsally along the carotid sheath. Intraoperatively, the ibCLN was lifted away from the lateral surface of the pharynx through the traction of the tissue retractor in all the specimens. Our findings, therefore, provide evidence to disprove previous contentions that the ibCLN may be injured during the dissection of the septum of the caudal pharyngeal constrictor muscles or the placement of the prosthesis through the muscular process.^{11,13}

The perforation of the cricoid cartilage with the Deschamps needle and the tying of the prosthesis seemed to play a marginal role for possible intraoperative neural damage.

4.3 | Clinical relevance of the damages to the vagal branches

Iatrogenic neural damage causing dysphagia is supposed to occur unilaterally during one-sided laryngoplasty. Unfortunately, the consequences of unilateral impairment of the branches of PP in horses are unknown. On the other hand, bilateral and unilateral suppressions of the vagal and glossopharyngeal components of the PP have been studied. Dysphagia has been reported after bilateral and unilateral regional anesthesia of the pharyngeal branch of the vagus nerve^{33,34} and in unilateral mononeuropathy of the vagus nerve in guttural pouch mycosis.³⁵ Barakzai et al.⁹ described a case of bilateral nasal discharge of food one month after laryngoplasty. The postmortem examination revealed gross and histological changes of the region innervated by the PPes.9,22 These findings may be explained by intraoperative damage to the PPes and other branches of the PP containing motor fibers. In contrast, bilateral regional anesthesia of the glossopharyngeus nerve was not associated with disturbances of the pharyngeal phase of swallowing.36 Therefore, we suggest that intraoperative damage to branches of PP innervating the caudal pharyngeal constrictor muscles and the cervical esophagus may lead to dysphagia after laryngoplasty. For this reason, every effort should be made to visualize the branches of the PP and to treat them with care during surgery.

While bilateral regional anesthesia of the ibCLN in man resulted in dysphagia, coughing, and silent aspiration,³⁰ unilateral impairment of ibCLN has rarely been associated with swallowing dysfunction.²⁹ Similarly, unilateral neurectomy of the ibCLN did not result in dysphagia in horses and dogs.^{24,37} Sensory loss in the ipsilateral soft palate and pharyngolaryngeal mucosa was reported by Gaughan et al.²⁴ Although tactile stimulation of the ipsilateral mucosa failed to adduct the arytenoid

cartilage and to elicit the swallowing reflex, short-term (up to seven days) coughing or dysphagia were not reported. Therefore, unilateral severing of the CLN during laryngoplasty may not be sufficient to produce swallowing disorders.

Iatrogenic trauma to the ebCLN may lead to cricothyroid muscle atrophy, which has been suggested to cause palatal and vocal fold instability but not dysphagia after laryngoplasty.^{17,26} In the present study, the ebCLN also innervated the thyropharyngeal muscle in one of the specimens, so damage to the ebCLN may result in partial atrophy of the thyropharyngeal muscle in corresponding individuals.

In summary, the neuroanatomy of the equine pharyngeal and laryngeal regions may be more complex and variable than what has been previously described. Similarly, neural communications between PP and CLN resulting in mixed nerve branches have recently been reported in man and in pigs.^{28,38-42} This neuronal variability may explain the absence of clinical implications after a single nerve injury. Moreover, individual variation in the neuronal pattern may explain the diversity of the clinical signs and the discrepancy between the relatively moderate incidence of severe clinical signs after laryngoplasty despite the common exposure of the vagal branches to intraoperative injury as highlighted in this study.

The main limitations of this study were the limited number of specimens and their exsanguination, which might have minimally influenced the topographical relationships. Although the use of head-neck specimens may be associated with individual variation in the position of the cranial nerves despite the accurate fixation of the trachea, no appreciable differences in the neuroanatomy between the whole cadavers and head-neck specimens were identified. Moreover, the rate of damage may even be underestimated in this study because it could be appreciated only in the specimens in which the nerves were identified within the surgical field.

This study expands the anatomical knowledge of the surgical field of laryngoplasty in horses and provides evidence of the plausibility of intraoperative injuries to the ibCLN, ebCLN, and PP. Moreover, a branch of the ebCLN innervating the thyropharyngeal muscle was described for the first time in horses, highlighting the still underestimated variability of the neuroanatomy in this species. This study revealed that the branches of the PP (PPcr, PPth, PPes) and the CLN (ibCLN, ebCLN) are all at risk for iatrogenic injury during prosthetic laryngoplasty. Therefore, early identification of the branches of the vagus nerve and careful surgical exploration of the pharyngeal regions are mandatory to reduce the incidence of iatrogenic neural trauma and subsequent dysphagia. Neural structures should be carefully discriminated from

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lymphatic vessels that course with similar pathways. Another neuronal structure at risk during laryngoplasty (steps 1 and 7) is the first cervical nerve, which contributes to the ansa cervicalis (Figure 2B). Damage to this nerve may be expected to result in a dorsal displacement of the soft palate,⁴³ a complication which has also been reported in horses after laryngoplasty.⁴⁴

The manipulation of the caudal pharyngeal constrictor muscles should be minimized, and the vagus nerve must be spared when the tissue retractor is placed. The ebCLN should be carefully dissected and retracted rostrally over the caudal border of the thyropharyngeal muscle. Tissue retractor-related injury may be reduced by a brief and gentle use of a retractor with a short and broad blade to reduce and distribute its pressure on tissue enclosing to the described nerves. Moreover, the use of a narrow Langenbeck retractor might concentrate the pressure on a smaller area. However, additional research is required to define a better way to retract the tissue without damaging relevant structures.

Although valuable information may be drawn from these findings, additional studies are required to gather additional knowledge regarding the intersubjective variability of the innervation patterns and, with respect to the clinical bearing of our anatomical findings, to elucidate the impact of unilateral injury of the main branches of the PP and the CLN. Moreover, alternative surgical techniques (eg, laryngeal reinnervation⁴⁵) that are less harmful to the neurogenic structures of the pharyngeal and laryngeal regions should be further explored.

ACKNOWLEDGMENTS

AUTHOR CONTRIBUTIONS

Pisano SRR, MVetMed, Dr Med Vet, DECZM: Designed the study, prepared the specimens, performed the anatomical study, wrote the manuscript, and revised and accepted the final version of the submitted manuscript; Stoffel MH, Dr Med Vet Habil: Performed the anatomical study and revised and accepted the final version of the submitted manuscript; Bodó G, DVM, PhD, DSc, DECVS: Designed the study, prepared the specimens, performed the laryngoplasty, and revised and accepted the final version of the submitted manuscript.

The authors thank Karsten Hofmann (University of Bern) and Christoph Koch Dr Med Vet DACVS DECVS (University of Bern) for assistance with the preparation of the specimens; Daniela Casoni DVM DECVAA (University of Bern), Peter M. DiGeronimo DMV MSc DACZM (Louisiana State University), Maja A. Waschk DVM DECVDI (University of Bern) and Gaia A. Moore-Jones Med Vet PhD (University of Bern) for assistance with the preparation of the manuscript; and Aleksandar Panić for assistance with the preparation of the figures.

CONFLICT OF INTEREST

The authors declare no conflicts of interest related to this report.

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REFERENCES

- Ahern BJ, Parente EJ. Surgical complications of the equine upper respiratory tract. *Vet Clin North Am Equine Pract.* 2008; 24(3):465-484. https://doi.org/10.1016/j.cveq.2008.10.004.
- Ducharme NG, Rossignol F. Larynx. In: Auer JA, Stick JA, eds. *Equine Surgery*. 5th ed. Philadelphia, PA: Saunders Elsevier; 2019: 734-769. https://doi.org/10.1016/B978-0-323-48420-6.00046-6.
- Dixon RM, McGorum BC, Railton DI, et al. Long-term survey of laryngoplasty and ventriculocordectomy in an older, mixed-breed population of 200 horses. Part 1: maintenance of surgical arytenoid abduction and complications of surgery. *Equine Vet J.* 2003; 35(4):389-396. https://doi.org/10.2746/042516403776014172.
- Hawkins JF, Tulleners EP, Ross MW, Evans LH, Raker CW. Laryngoplasty with or without ventriculectomy for treatment of left laryngeal hemiplegia in 230 racehorses. *Vet Surg.* 1997;26(6): 484-491. https://doi.org/10.1111/j.1532-950x.1997.tb00521.x.
- Strand E, Martin GS, Haynes PF, McClure R, Vice D. Career racing performance in Thoroughbreds treated with prosthetic laryngoplasty for laryngeal neuropathy: 52 cases (1981-1989). *J Am Vet Med Assoc.* 2000;217(11):1689-1696. https://doi.org/ 10.2460/javma.2000.217.1689.
- Barakzai SZ, Barnett TP, Dixon PM. Oseophageal reflux following prosthetic laryngoplasty. In: Proceedings of the European College of Veterinary Surgeons 22nd Annual Scientific Meeting; July 4-6, 2013; Rome, Italy.
- Barnett TP, O'Leary JM, Parkin TD, Dixon PM, Barakzai SZ. Long-term exercising video-endoscopic examination of the upper airway following laryngoplasty surgery: a prospective cross-sectional study of 41 horses. *Equine Vet J.* 2013;45(5):593-597. https://doi.org/10.1111/evj.12020.
- Leutton JL, Lumsden JM. Dynamic respiratory endoscopic findings pre- and post laryngoplasty in Thoroughbred racehorses. *Equine Vet J.* 2015;47(5):531-536. https://doi.org/10. 1111/evj.12331.
- Barakzai SZ, Dixon PM, Hawkes CS, Cox A, Barnett P. Upper esophageal incompetence in five horses after prosthetic laryngoplasty. *Vet Surg.* 2015;44(2):150-155. https://doi.org/10. 1111/j.1532-950X.2014.12101.x.
- Goulden BE, Anderson LG. Equine laryngeal hemiplegia. Part III. Treatment by laryngoplasty. N Z Vet J. 1982;30(1-2):1-5.
- 11. Adreani CM, Parente EJ. Surgical treatment of laryngeal hemiplegia and hemiparesis. In: McGorum BC, Dixon PM, Robinson EN, Schumacher J, eds. *Equine Respiratory Medicine*

and Surgery. London, United Kingdom: Saunders Elsevier; 2007: 497-504. https://doi.org/10.1016/B978-0-7020-2759-8.50040-4.

- 12. Greet TR, Baker GJ, Lee R. The effect of laryngoplasty on pharyngeal function in the horse. *Equine Vet J.* 1979;11:153-158. https://doi.org/10.1111/j.2042-3306.1979.tb01328.x.
- 13. Ahern TJ. Laryngo-pharyngeal desensitization following a prosthetic laryngoplasty. *J Equine Vet Sci.* 1996;16:120-122.
- Fitzharris LE, Lane JG, Allen KJ. Outcomes of horses treated with removal of a laryngoplasty prosthesis. *Vet Surg.* 2019;48 (4):465-472. https://doi.org/10.1111/vsu.13150.
- 15. Velde K. Unusual complication after prosthetic laryngoplasty and vocal cordectomy in a horse. In: Proceedings of the European College of Veterinary Surgeons 19th Annual Scientific Meeting; July 1-3; 2010; Helsinki, Finland.
- Vidovic A, Delling U. Aryepiglottic fold augmentation as treatment for late-onset dysphagia following surgical treatment of recurrent laryngeal neuropathy. *Tierarztl Prax Ausg G Grosstiere Nutztiere*. 2017;45(4):219-225. https://doi.org/10. 15653/TPG-160712.
- Barnett TP, O'Leary JM, Dixon PM, Barakzai SZ. Characterisation of palatal dysfunction after laryngoplasty. *Equine Vet J.* 2014;46:60-63. https://doi.org/10.1111/evj.12081.
- Huskamp B. Komplikationen nach Kehlkopfoperationen. Prakt Tierartz, 1980;10:848-858.
- 19. Stoffel MH. Funktionelle Neuroanatomie für die Tiermedizin. Stuttgart, Germany: Enke; 2011.
- Sisson S, Grossman JD, Getty R. Sisson and Grossman's The anatomy of the domestic animals. 5th ed. Philadelphia, PA: WB Saunders; 1975.
- 21. Barone R, Simoens P. Neurologie II: Système nerveux périphérique, glandes endocrines, esthésiologie. Paris, France: Vigot; 2010.
- 22. Nomura S, Mizuno N. Central distribution of efferent and afferent components of the cervical branches of the vagus nerve. A HRP study in the cat. *Anat Embryol (Berl)*. 1983;166(1):1-18. https://doi.org/10.1007/bf00317941.
- 23. Quinlan TJ, Goulden BE, Barnes GR, Anderson LJ, Cahill JI. Innervation of the equine intrinsic laryngeal muscles. *N Z Vet J.* 1982;30:43-45.
- Gaughan EM, Hackett RP, Ducharme NG, Rakestraw PC. Clinical evaluation of laryngeal sensation in horses. *Cornell Vet.* 1990;80(1):27-34.
- 25. Brandenberger O, Martens A, Robert C, et al. Anatomy of the vestibulum esophagi and surgical implications during prosthetic laryngoplasty in horses. *Vet Surg.* 2018;47(7):942-950. https://doi.org/10.1111/vsu.12944.
- Holcombe SJ, Rodriguez K, Lane J, Caron JP. Cricothyroid muscle function and vocal fold stability in exercising horses. *Vet Surg.* 2006;35(6):495-500. https://doi.org/10.1111/j.1532-950X.2006.00182.x.
- Gutierrez S, Iwanga J, Pekala P, et al. The pharyngeal plexus: an anatomical review for better understanding postoperative dysphagia. *Neurosurg Rev.* 2020. https://doi.org/10.1007/ s10143-020-01303-5105
- Uludag M, Aygun N, Isgor A. The functional role of the pharyngeal plexus in vocal cord innervation in humans. *Eur Arch Otorhinolaryngol.* 2017;274(2):1121-1128. https://doi.org/10. 1007/s00405-016-4369-7.

- Paraskevas GK, Raikos A, Ioannidis O, Brand-Saberi Beate. Topographic anatomy of the internal laryngeal nerve: surgical considerations. *Head Neck*. 2012;34(4):534-540. https://doi.org/ 10.1002/hed.21769
- Shin DU, Sung JK, Nam KH, Cho DC. Bilateral internal superior laryngeal nerve palsy of traumatic cervical injury. *J Korean Neurosurg Soc.* 2012;52(3):264-266. https://doi.org/10.3340/jkns.2012.52.3.264.
- López A-JV, González Quarante LH, de Sagredo del Corral OLG, Montalvo Alfonso A, Fernández Carballal C. Hypoglossal nerve paresis secondary to anterior approach of upper cervical spine followed by spontaneous recovery. *J Spine Surg.* 2017;3(3):481-483. https://doi.org/10.21037/jss.2017.06.19.
- Lee HC, Kim HD, Park WK, Rhee HD, Kim KJ. Radial nerve paralysis due to Kent retractor during upper abdominal operation. *Yonsei Med J.* 2003;44(6):1106-1109. https://doi.org/10. 3349/ymj.2003.44.6.1106.
- Holcombe SJ, Derksen FJ, Stick JA, Robinson NE. Effect of bilateral blockade of the pharyngeal branch of the vagus nerve on soft palate function in horses. *Am J Vet Res.* 1998;59(4): 504-508.
- Virgin JE, Holcombe SJ, Caron JP, et al. Laryngeal advancement surgery improves swallowing function in a reversible equine dysphagia model. *Equine Vet J.* 2016;48(3):362-367. https://doi.org/10.1111/evj.12430.
- 35. Eichentopf A, Snyder A, Recknagel S, Uhlig A, Waltl V, Schusser GF. Dysphagia caused by focal guttural pouch mycosis: mononeuropathy of the pharyngeal ramus of the vagal nerve in a 20-year-old pony mare. *Ir Vet J.* 2013;66(1):13. https://doi.org/10.1186/2046-0481-66-13.
- Klebe EA, Holcombe SJ, Rosenstein D, Boruta D, Bartner LR, Tessier C. The effect of bilateral glossopharyngeal nerve anaesthesia on swallowing in horses. *Equine Vet J.* 2005;37(1):65-69. https://doi.org/10.2746/0425164054406900.
- Venker-van Haagen AJ, Van den Brom WE, Hellebrekers LJ. Effect of superior laryngeal nerve transection on pharyngeal muscle contraction timing and sequence of activity during eating and stimulation of the nucleus solitarius in dogs. *Brain Res Bull.* 1999;49(6):393-400. https://doi.org/10.1016/s0361-9230 (99)00067-2.
- Martin-Oviedo C, Maranillo E, Lowy-Benoliel A, et al. Functional role of human laryngeal nerve connections. *Laryngoscope*. 2011;121(11):2338-2343. https://doi.org/10.1002/lary.22340.
- Sañudo JR, Maranillo E, León X, Mirapeix RM, Orús C, Quer M. An anatomical study of anastomoses between the laryngeal nerves. *Laryngoscope*. 1999;109(6):983-987. https:// doi.org/10.1097/00005537-199906000-00026.
- Matsuzaki H, Paskhover B, Sasaki CT. Contribution of the pharyngeal plexus to vocal cord adduction. *Laryngoscope*. 2014;124 (2):516-521. https://doi.org/10.1002/lary.24345.
- Paskhover B, Wadie M, Sasaki CT. The pharyngeal plexusmediated glottic closure response and associated neural connections of the plexus. *JAMA Otolaryngol Head Neck Surg.* 2014;140(11):1056-1060. https://doi.org/10.1001/jamaoto.2014. 2440.
- 42. Woo JS, Hundal JS, Sasaki CT, Abdelmessih MW, Kelleher SP. Reflex vocal fold adduction in the porcine model: the effects of stimuli delivered to various sensory nerves. *Ann Otol Rhinol*

Laryngol. 2008;117(10):749-752. https://doi.org/10.1177/000348940811701008.

- Ducharme NG, Hackett RP, Woodie JB, et al. Investigations into the role of the thyrohyoid muscles in the pathogenesis of dorsal displacement of the soft palate in horses. *Equine Vet J.* 2003;35 (3):258-563. https://doi.org/10.2746/042516403776148200.
- 44. Barnett TP, O'Leary JM, Dixon PM, Barakzai SZ. Characterisation of palatal dysfunction after laryngoplasty. *Equine Vet J*. 2014;46(1):60-63. https://doi.org/10.1111/evj.12081.
- 45. Rossignol F, Brandenberger O, Perkins JD, Marie J-P, Mespoulhès-Rivère C, Ducharme NG. Modified first or second cervical nerve transplantation technique for the treatment of

recurrent laryngeal neuropathy in horses. *Equine Vet J.* 2018;50 (4):457-464. https://doi.org/10.1111/evj.12788.

How to cite this article: Pisano SRR, Stoffel MH, Bodó G. Ex vivo study of vagal branches at risk for iatrogenic injury during laryngoplasty in horses. *Veterinary Surgery*. 2020;1–10. <u>https://doi.org/10.</u> <u>1111/vsu.13548</u>