AN EX VIVO MODEL TO EVALUATE THE EFFECT OF CYCLICAL ADDUCTORY FORCES ON MAINTENANCE OF ARYTENOID ABDUCTION AFTER PROSTHETIC LARYNGOPLASTY PERFORMED WITH AND WITHOUT MECHANICAL ARYTENOID ABDUCTION

THESIS

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Abstract

Left laryngeal hemiplegia is a major reason for exercise intolerance and respiratory noise in performance horses. Prosthetic laryngoplasty is the current standard for treating left laryngeal hemiplegia, but has a failure rate in performance horses of 30-50%. Failure is due to loss of abduction of the left arytenoid cartilage, and tearing of the prosthetic suture through the cartilage is considered to be a major cause of abduction failure. This loss of arytenoid abduction begins within days of surgery, and little is understood about the forces that cause this loss of abduction, and how to prevent it. One possible contributor is the considerable tension that must be put on the prosthetic suture used to abduct the arytenoid. This force, and suture abrasion through the cartilage, may play a role in the initiation of suture pull-through.

Currently, most investigations of laryngoplasty failure use a large magnitude tensile force in a single cycle to failure model. Single cycle models fail to address a potential major cause of early abduction failure, which are the cyclical adduction forces on the arytenoid cartilage associated with swallowing and coughing. The primary goal of this study is to develop a model using cadaveric larynges that applies cyclical adductory forces on the arytenoid similar to those that may be generated during swallowing or coughing and to determine if these forces cause a loss of abduction comparable to that reported in horses within the first week after laryngoplasty. A secondary goal is to determine if using a clamp to abduct the arytenoid cartilage prior to knot tying will
improve maintenance of the abduction when subjected to the cyclical adductory forces of this model.

Larynges from horses aged 2 - 4 years (median 3.4) were used. Left arytenoid laryngoplasty was performed using a single suture of #5 Ethibond with (n=7) and without (n=7) abducting the left arytenoid with a clamp before knot tying. Each laryngoplasty was tested under cyclic loading of 2 to 26 N at 0.5 Hz for 5000 cycles. Arytenoid displacement data were collected at 1 Hz intervals and analyzed using commercial statistical software [Microsoft Excel, Microsoft Corp., Redmond, WA and GraphPad Prism v5 GraphPad Software, La Jolla, CA]. Comparisons between groups (clamped and non-clamped larynges) and between cycling intervals were assessed using a Mann-Whitney U test. Comparisons at different cycling intervals were assessed using a Friedman statistic for repeated measures with Dunn’s post hoc comparison testing for each group of larynges. Significance was set at P < 0.05.

Median left arytenoid abduction distance was 16.9 mm (range 9.8-19.8 mm). One larynx in each group failed at < 1000 cycles. There was no difference (p=0.13) in the percentage loss of left arytenoid abduction between clamped 46.2% (7.6 mm) and non-clamped larynges 31.9% (5.2 mm) after 5000 cycles. This model of cyclical adduction resulted in arytenoid displacements similar to those occurring in the first week after surgery.

Ex vivo cyclical adductory forces produced a significant loss of laryngoplasty abduction. The use of a clamp to abduct the arytenoid cartilage prior to knot tying did not reduce the loss of abduction. Cyclical adductory forces after surgery may be important in
early loss of laryngoplasty abduction. This model should be useful in testing novel techniques of laryngoplasty.
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Chapter 1: Introduction

Left laryngeal hemiplegia (LLH) is one of the major reasons for exercise intolerance and respiratory noise in performance horses.\textsuperscript{1-3} The prevalence of laryngeal hemiplegia in Thoroughbreds is between 2-10\%, and in some Draft breeds has been suggested to exceed 40\%.\textsuperscript{2-6} Currently, equine laryngeal paralysis is an idiopathic disease that results in neurogenic atrophy of the left laryngeal intrinsic musculature due to left recurrent laryngeal nerve dysfunction. The left recurrent laryngeal nerve branches from the left vagus nerve at the level of the base of the heart, curves medially around the aorta and ligamentum arteriosum to lie on the lateral wall of the trachea as it courses cranially to the laryngeal musculature.\textsuperscript{7} In contrast, the much shorter right recurrent laryngeal nerve branches from the vagus at the level of the first intercostal space and passes medially around either the right subclavian or costocervical artery before it joins up with the lateral wall of the trachea and heads towards the larynx. The intrinsic laryngeal musculature affected in LLH includes laryngeal adductors: cricoarytenoideus lateralis, thyroarytenoideus, vocalis, ventricularis, arytenoideus transversus, and laryngeal abductors, cricoarytenoideus dorsalis and cricothyroideus. The adductor muscles are the first to be affected and therefore show the most severe damage.\textsuperscript{8-15}

The cause of LLH is not known. It is proposed that the left side is affected more frequently due to its greater length. Proposed etiologies for left laryngeal paralysis include: mechanical stretching of the recurrent laryngeal nerve during neck movements.\textsuperscript{16}
environmental toxins or vitamin deficiencies, generalized neurological dysfunction\textsuperscript{17, 18}. However none of these proposed etiologies have been definitively shown to result in laryngeal paralysis of the horse. Similar to the acquired form of laryngeal paralysis in the dog, equine laryngeal paralysis can also occur as a result of trauma or neoplasia. Histopathologic lesions associated with equine laryngeal paralysis have been characterized as a distal axonopathy with a decrease in large myelinated fibers that are more severe in the distal portions of the left recurrent laryngeal nerve\textsuperscript{9,10,14,19,20}. Although equine left laryngeal hemiplegia appears to be hereditary the characteristic mononeuropathic nature of the disease makes it unique from inherited myelinopathies and axonopathies in other species in that only one nerve is affected in the horse\textsuperscript{2,21-24}. Dog breeds that are reported to have a hereditary congenital form of laryngeal paralysis include: dalmatians, rottweilers, bouviers, huskies (husky crossbreeds), bull terriers, white coated German shepherds, Leonbergers, and Pyrenean mountain dogs\textsuperscript{25-31}. In bouviers the condition is transmitted in an autosomal dominant pattern and is characterized by a loss of motor neurons in the nucleus ambiguus with secondary Wallerian degeneration of the recurrent laryngeal nerve\textsuperscript{32}. In huskies and white-coated German shepherds heritability is thought to be linked with their white coat color that results in depletion of motor neurons in the nucleus ambiguus\textsuperscript{29,30,33}. An autosomal recessive mode of inheritance has been proposed for laryngeal paralysis in Pyrenean mountain dogs based on pedigree analysis\textsuperscript{31}. Dalmatians, rottweilers, and Pyrenean mountain dogs have all been reported to have a laryngeal paralysis-polyneuropathy complex resulting in neurogenic atrophy of intrinsic laryngeal muscles and appendicular
skeletal muscles\textsuperscript{26-28,31}. X-linked recessively inherited polyneuropathy and laryngeal paralysis has also been reported in adult Leonberger dogs that may be similar to human Charcot-Marie-Tooth neuropathy\textsuperscript{34}. Horses affected with LLH do not show clinical signs of polyneuropathy such as megaoesophagus, bilateral stringhalt, tetraparesis or muscle atrophy as is observed in dogs with some forms of LLH\textsuperscript{26}. The lesions characterized in dogs as loss of motor neurons in the nucleus ambiguus with secondary Wallerian degeneration of the recurrent laryngeal nerve have not been confirmed in the horse following extensive histological examinations of the brains of LLH affected horses\textsuperscript{35}. Pathological changes have been reported in other long peripheral nerves in the horse (common, deep and superficial peroneal and tibial nerves) as well as neurogenic atrophy of the long digital extensor muscle in some LLH affected horses\textsuperscript{36} but these findings were made without a proper control group, and do not take into account that age-related pathological changes occur in distal limb nerves of older horses unaffected by LLH\textsuperscript{37}.

The diagnosis of LLH can be made through a combination of a history, physical exam, and endoscopy of the upper airway. Horses with laryngeal paralysis commonly present with a history of inspiratory noise ("roaring" or "whistling") during exercise and poor performance. Although equine laryngeal paralysis is most commonly the result of a recurrent laryngeal neuropathy, other potential causes such as 4\textsuperscript{th} branchial arch defect, arytenoid chondritis, external or iatrogenic related trauma to the recurrent laryngeal nerve, or neoplasia can be ruled in or out with a thorough history and good physical exam\textsuperscript{38–42}. The history should include information regarding when respiratory noise occurs (inspiration, expiration, or both), does the occurrence correspond to exercise
intensity, results of previous endoscopic examination, the effect of head position on the magnitude or presence of noise, is there a decrease in performance, has prior upper airway surgery been performed, and has the horse had any evidence/history of lower respiratory or cardiac disease that may result in decreased performance\textsuperscript{2,38}.

Physical exam is extremely important in diagnosing laryngeal paralysis as the cause for upper respiratory noise and exercise intolerance or poor performance in the horse. Thorough auscultation of the heart and lungs should be performed to rule out any cardiac or lower respiratory abnormalities\textsuperscript{2,38}. During the physical one should determine if the noise occurs during inspiration, expiration, or both through careful auscultation of both phases of respiration\textsuperscript{2,38}. Equine laryngeal paralysis causes turbulence and obstruction during inspiration, so the presence of obstruction during expiration may indicate an abnormality unrelated to laryngeal paralysis. The amount of airflow from each nostril should be evaluated to look for evidence of nasal passage obstruction which may produce inspiratory noise despite normal laryngeal function\textsuperscript{2,38}. One should perform careful palpation/evaluation of the head and neck regions looking for evidence of external trauma or iatrogenic associated trauma that can lead to thrombophlebitis, peri-venous obstruction, and damage to either the left or right recurrent laryngeal nerve along with laryngeal paralysis of the affected side\textsuperscript{38,42}. Horner’s syndrome may also be associated with trauma to these areas as a result of damage to the sympathetic nerve. Horses with Horner’s syndrome may show signs of sweating on the neck and base of the ear of the affected side, as well as ptosis, myosis, and enophthalmos\textsuperscript{43,44}. Careful palpation of the larynx can reveal muscular atrophy of the cricoarytenoideus dorsalis which is evident by
a more pronounced arytenoid muscular process on the side affected by laryngeal paralysis. A 4th branchial arch defect as the cause of laryngeal dysfunction can be palpated as an abnormal space between the thyroid and cricoid cartilages, although the right side is more commonly affected than the left.

Video endoscopic examination of the upper airway is currently the most common a widely excepted diagnostic technique for equine laryngeal paralysis. Endoscopy of the upper airway can be performed in the standing horse at rest, during high speed treadmill exercise, and more recently during exercise under saddle. High speed treadmill endoscopy is considered the gold standard for the evaluation of the upper equine respiratory tract, however most practitioners perform endoscopy at rest because of the high financial cost and extra time/personal requirement associated with high speed treadmill endoscopy. While standing endoscopy is good for morphological rule out of other disorders of the larynx it can miss subclinical disease that will only be apparent during exercise.

Numerous grading scales have been proposed for the endoscopic evaluation of laryngeal function at rest. The most widely accepted system is the Havemeyer scale, a four grade evaluation of laryngeal function in the standing unsedated horse. Subgrades are added to further differentiate grades 2 and 3. Grade 1 is defined by synchronous and symmetrical movements of the arytenoid cartilages along with the ability to achieve and maintain full arytenoid cartilage abduction. Grade 2 is arytenoid cartilage movements that are asynchronous or when the larynx is asymmetric intermittently, but full abduction can be achieved and maintained. Grade 3 is asynchronous/asymmetrical cartilage
movements without the ability to achieve and maintained full abduction. Grade 4 is defined as complete paralysis of the arytenoid cartilage and vocal fold\textsuperscript{49} (Table 1).

Table 1: Havemeyer Grading System

<table>
<thead>
<tr>
<th>Grade</th>
<th>Description</th>
<th>Sub-grade</th>
</tr>
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<tbody>
<tr>
<td>I</td>
<td>All arytenoid cartilage movements are synchronous and symmetrical and full arytenoid cartilage abduction can be achieved and maintained</td>
<td>.1 Transient asynchrony, flutter or delayed movements are seen. .2 There is asymmetry of the rima glottidis much of the time due to reduced mobility of the affected arytenoid and vocal fold but there are occasions, typically after swallowing or nasal occlusion when full symmetrical abduction is achieved and maintained.</td>
</tr>
<tr>
<td>II</td>
<td>Arytenoid cartilage movements are asynchronious and/or larynx asymmetric at times but full arytenoid cartilage abduction can be achieved and maintained.</td>
<td>.1 There is asymmetry of the rima glottidis much of the time due to reduced mobility of the arytenoid and vocal fold but there are occasions, typically after swallowing or nasal occlusion when full symmetrical abduction is achieved but not maintained. .2 Obvious arytenoid abductor deficit and arytenoid asymmetry. Full abduction is never achieved. .3 Marked but not total arytenoid abductor deficit and asymmetry with little arytenoid movement. Full abduction is never achieved.</td>
</tr>
<tr>
<td>III</td>
<td>Arytenoid cartilage movements are asynchronious and/or asymmetric. Full arytenoid cartilage abduction cannot be achieved and maintained.</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>Complete immobility of the arytenoid cartilage and vocal fold</td>
<td></td>
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</table>

Endoscopy performed during high speed treadmill exercise is the current ‘gold standard’ for the evaluation of the upper respiratory tract. However, in the horse studies have shown that roughly 90% of horses categorized as grade 3 during resting endoscopy
will have an abnormal exam during treadmill exercise, and about 10% will have normal laryngeal function\textsuperscript{48,50,51}. Similarly, 90% of horses categorized with normal laryngeal function at rest grades 1 and 2 will be normal during treadmill examination; and 10% will demonstrate abnormal function\textsuperscript{50,51}. The potential to misclassify horses examined at rest is why endoscopy during high speed treadmill exercise is considered superior to resting endoscopy. Recently, the ability to examine horse exercising over ground using dynamic respiratory endoscopy technology has become available. Over ground examination theoretically allows the practitioner to examine the patient in more natural exercise conditions without the need for treadmill training sessions, experienced treadmill personnel, and the cost of a treadmill. Studies on the use of remote dynamic endoscopy to diagnose upper airway dysfunction have demonstrated the equipment to be safe and useful in diagnosing some of the same dysfunctions observed during treadmill endoscopy\textsuperscript{52,53}. However, at this time protocols are not as well established for remote examination as they are for treadmill examination which may produce more variability with remote endoscopy diagnostics\textsuperscript{54,55}.

Surgical treatments of left laryngeal hemiplegia include ventriculectomy / ventriculocordectomy (VC) alone, partial arytenoidectomy (PA), laryngeal reinnervation (LR), and prosthetic laryngoplasty (PLP) +/- ipsilateral VC +/- contralateral ventriculectomy. Ventriculectomy was first suggested by Professor Gunther of the veterinary school at Hanover in 1834, taken up again by Professor Williams at Cornell University, and was popularized by Hobday in 1935\textsuperscript{56-58}. The procedure involves
removal the more commonly affected left laryngeal ventricular saccule that was noted to collapse into the airway and restricts the ventral diameter of the larynx in horses affected with equine laryngeal paralysis\textsuperscript{59-61}. Initially, the procedure was thought to induce adhesions between the left arytenoid and thyroid cartilages which would limit axial displacement of the arytenoid cartilage during exercise. However subsequent studies have shown no measurable evidence that ventriculectomy alone yields any improvement in the size of the rima glottidis\textsuperscript{59-61}. Many surgeons still perform ventriculectomy in combination with either unilateral or bilateral cordectomy. The combination of a ventriculectomy and cordectomy (ventriculocordectomy) came about and is now a common practice, due to the benefit of improved airway function and noise reduction compared to ventriculectomy alone.

Research has demonstrated that both prosthetic laryngoplasty (discussed latter) and either unilateral vocal cordectomy with bilateral ventriculectomy or bilateral VC will reduce, but not eliminate, upper airway obstruction in laryngeal hemiplegia affected horses\textsuperscript{60}. A study comparing VC to PLP in treadmill exercised horses, found that both procedures fail to normalize blood gas parameters\textsuperscript{62}. It has also been shown that the addition of either unilateral vocal cordectomy with bilateral ventriculectomy or bilateral VC to PLP does not improve upper airway flow mechanics over prosthetic laryngoplasty alone\textsuperscript{61}. Several studies have demonstrated that PLP alone was inferior to VC in reducing abnormal upper airway noise associated with equine laryngeal paralysis\textsuperscript{63-65}. As a result of these studies, it is generally believed that horses performing a submaximal exercise which present for upper airway noise without poor performance or exercise
intolerance may be good candidates for VC alone\textsuperscript{45,51,63}. However, a large percentage of horses have been reported to make abnormal respiratory noises post VC (34\%) as compared to laryngoplasty combined with VC (27\%)\textsuperscript{66}. Therefore, a large percentage of surgeons prefer to us a combination of PLP and VC when treating equine laryngeal paralysis.

Another surgical treatment for LLH is removal of the affected arytenoid cartilage or arytenoidectomy. This procedure is used in cases of chronic arytenoid chondropathy, following failed PLP, in the presence of severe arytenoid cartilage ossification, or in some cases as a primary treatment for equine LLH. The procedure has been described using either a subtotal technique or partial arytenoidectomy (PA) technique. Subtotal arytenoidectomy (SA) involves removal of the arytenoid cartilage while sparing both the corniculate and muscular processes. PA incorporates removal of the corniculate process and body of the arytenoid cartilage, but spares the muscular process. Partial arytenoidectomy is the preferred technique based on evidence that the procedure significantly improves airway function and SA fails demonstrate any benefit to airway mechanics\textsuperscript{67-74}. Studies have shown that PA can be performed with or without mucosal closure and both techniques produce good results\textsuperscript{74-76}. When comparing PA to PLP with VC both procedures have been shown to restore airway ventilation to normal at submaximal exercise however, at maximal exercise PLP/VC was slightly superior to PA\textsuperscript{69}. Racing Thoroughbreds treated with either PLP/VC or PA have similar prognoses to return to racing however, partial arytenoidectomy leads to inferior earnings after surgery\textsuperscript{77}. Complications associated with PA include: granulation tissue, hematoma
formation, suture dehiscence (mucosal closure), and aspiration/dysphagia in 36% of horses\textsuperscript{70}.

The ideal and the most physiologically appealing treatment for equine laryngeal paralysis would be to restore the function of the cricoarytenoideus dorsalis muscle. However, several surgeons have attempted reinnervation procedures with minimal results\textsuperscript{78-83}. Reinnervation of the cricoarytenoideus dorsalis muscle involves taking a branch from the first cervical nerve and a block of muscle where the nerve inserts on the omohyoideus muscle as a graft and then inserting the graft into pockets of the affected cricoarytenoideus dorsalis\textsuperscript{78-83}. Studies on the ideal anatomical location to place the neuromuscular grafts within the cricoarytenoideus dorsalis have suggested that the lateral compartment may be more beneficial than the medial muscle compartment to maximize abduction\textsuperscript{84,85}. In a study on both raced and unraced thoroughbreds: 95% (56/59) of previously raced and 60% (39/66) unraced started one or more races following neuromuscular pedicle graft. The previously raced horses in this study had an average time to first race of 7.5 and 8.6 months for Grade 3 and Grade 4 laryngeal function respectively, whereas the unraced horses had their first race at an average age of 3.1 years with an average of 10.6 starts. This study also showed that the money earned in the unraced group was consistent with the national average earnings for horses of the same age\textsuperscript{83}. The biggest disadvantage is time from surgery to the first race which makes this technique more appropriate for yearlings and early two year olds due to the typically short length of race careers\textsuperscript{83,56}.
Prosthetic laryngoplasty as first described by Marks et al. in 1970, in combination with ipsilateral vocalcorderctomy and unilateral or bilateral ventriculectomy remains the current standard of surgical treatment for equine laryngeal paralysis. Variations in the original technique have been described, but the general goal of all techniques is to prevent collapse of the arytenoid cartilage into the laryngeal lumen during exercise by placing a prosthesis, usually consisting of one or two strands of nonabsorbable braided suture material between the caudal edge of the cricoid cartilage and the muscular process of the arytenoid cartilage to simulate the action of the cricoarytenoideus dorsalis muscle\textsuperscript{1,65,77,86-91,95-97} Surgical complications following prosthetic laryngoplasty can be divided into both short term and long term complications. Short term surgical complications and reported rates include excessive loss of laryngoplasty abduction requiring a second surgery 5%, dysphagia 4% - 43%, coughing 4% - 30%, incisional problems 3% - 23% (seromas, abscess, drainage, dehiscence), and mucosal granuloma formation on the corniculate process 1% \textsuperscript{65,84,86,87,89,91-94}. Long term surgical complications and reported rates include loss of laryngoplasty abduction 5%, persistent dysphagia 3% - 13%, persistent coughing 3% - 57%, and incisional problems 4% \textsuperscript{65,84,86,87,89,91-94}. The success rate of laryngoplasty in the racehorse based on varied performance measurements is reported to be 38-78\% \textsuperscript{65,77,86,87,91,95-97} and is up to 91\% in horses performing in other disciplines\textsuperscript{98,99}. The loss of arytenoid abduction reported postlaryngoplasty occurs largely within the first week after surgery\textsuperscript{92}. The high incidence of laryngoplasty failure in the racehorse warrants further investigation into the mechanisms behind early loss of abduction.
Laryngoplasty suture pull-through in either the cricoid or arytenoid cartilage is considered a major cause of progressive loss of abduction, however there is no agreement as to why, how or where it occurs. Experimentally, prosthesis material, suture configuration, suture placement site, and implantation techniques all play a role in failure; however the impact of cartilage age on loss of abduction remains unclear. Several researchers have suggested that damage to either the arytenoid or cricoid cartilages during the initial placement of a prosthesis by the needle or suture itself during knot tying may initiate suture pull-through and loss of laryngoplasty abduction. A recent in vivo study measured the force transferred to the cartilage from the suture during knot tying at a mean of 27.6 ± 3.1 Newtons (N) (range 23.1 – 44.3 N) to achieve optimal intraoperative arytenoid abduction. We hypothesized that abducting the cartilage by a method other than the suture would avoid the initiation of cartilage damage at the puncture sites when the suture is used to abduct the arytenoid. A purpose-made clamp was designed and constructed to abduct the left arytenoid after suture placement so the knot was tied with less force and potential damage to the cartilages. In addition to damage that may occur during the placement of a laryngoplasty prosthesis, many researchers also believe that cyclic forces in the immediate postoperative period associated with coughing and swallowing may play an important role in the pathogenesis of post-laryngoplasty loss of abduction.

Although most in vitro studies of laryngoplasty failure used single cycle to failure, cyclical forces using either a vacuum system to mimic inspiratory flow rates during exercise or a servohydraulic testing machine on disarticulated laryngeal
cartilages have been reported. To further investigate the impact of early postoperative cyclical adductory forces on abduction, we developed a model that attempts to replicate these forces. As intrinsic adduction of the left arytenoid is severely compromised in horses affected with laryngeal hemiparesis, adductory forces on an abducted arytenoid must come largely from unaffected extrinsic laryngeal muscles. The mechanics of equine swallowing are poorly understood, however, swallowing has been proposed to cause medioventral pressure on the abducted arytenoid, and has been shown to increase the force on the laryngoplasty suture from rest by up to 10 times. We propose that the cricopharyngeus acts as a major part of the upper esophageal sphincter in the horse, as in other species. The fibers of both pharyngeal muscles and the dense intermuscular septum that connects them on their long axis are oriented perpendicularly to the long axis of the spine of the arytenoid muscular process and we believe that contraction of these muscles contributes an adductory force to the muscular process of the arytenoid during swallowing or coughing. Our model attempts to replicate the direction of this force.

The primary goal of this study is to develop a model using cadaveric larynges that applies cyclical adductory forces on the arytenoid similar to those that may be generated by the intermuscular septum during swallowing or coughing and to determine if these forces cause a loss of abduction comparable to that reported in horses within the first week after laryngoplasty. Our second goal was to use this model to test that hypothesis that using a clamp to abduct the arytenoid cartilage prior to knot tying will improve maintenance of prosthetic arytenoid abduction when subjected to the cyclical adductory forces of this model.
Chapter 2: Materials and Methods

Larynges were collected from horses euthanized for reasons unrelated to respiratory dysfunction. Specimens were preserved in a 2% phenoxyethanol solution and stored at -29 °C. After thawing, the *cricoarytenoideus dorsalis* and *arytenoideus transversus* muscles were removed from the left hemilarynx to facilitate prosthesis placement and mimic the muscle atrophy seen in laryngeal hemiplegia.

Larynges were randomly assigned to one of two groups (non-clamped and clamped) using a random number generator. In the non-clamped larynx group, left laryngoplasties were performed with a single suture prosthesis in a standard fashion. Briefly, using a single polyethylene terephthalate suture [#5 Ethibond, Ethicon, Somerville, NJ] the cricoid cartilage was penetrated by a #3 non-swedged trocar point half-circle needle [Anchor needle, Addison, IL] 1.5 cm from the caudal aspect of the cricoid cartilage and 5 mm lateral to the dorsal midline. A 0 trocar-point half circle needle was used to penetrate the arytenoid cartilage at the caudoaxial aspect of the cricoarytenoid joint 1 cm from the caudal edge of the muscular process. The needle was directed in a craniolateral direction through the arytenoid facet of the cricoarytenoid joint to include the spine of the muscular process in the tied suture. The caudal aspect of the suture engaged the midline notch in the cricoid cartilage. The degree of left arytenoid abduction was assessed visually with the goal of achieving a Dixon grade 2 abduction.
prior to mounting and testing. The sutures were tied using a surgeon’s knot followed by 4 single throws (Figure 1).

![Image of larynx after laryngoplasty with labels for Cricoid, Muscular Process of Arytenoid, and Suture]

**Figure 1** – Photograph of dorsal aspect of non-clamped larynx after laryngoplasty

In the clamped larynx group, left laryngoplasties were performed similarly, but the left arytenoid cartilage was abducted with a clamp (Figure 2) after suture placement and before knot tying.
To estimate larynx size, the length of the left arytenoid from apex to base was measured. A modification of a previously reported method for quantifying abduction distance after laryngoplasty was used in this experiment\textsuperscript{85}. Markers were placed on the base of both corniculate processes and at the mid-point between the apex and base of the
left corniculate process (Figure 3a). Left arytenoid position was determined by measuring the distance between the base of the right arytenoid with 1) the base and 2) the middle of left arytenoid, and calculating the average. After laryngoplasty was performed measurements were repeated (Figure 3b), and the left arytenoid abduction distance was determined by subtracting the pre-abduction position from the post-abduction position.

**Figure 3** – Photograph of rostral larynx before laryngoplasty (A) and after (B) with biomarkers placed at the base of both corniculate processes and a marker placed in the middle of the left arytenoid. Left arytenoid abduction distance formula: Abduction = \((A2 + B2 / 2) - (A1 + B1 / 2)\)
Photographs with size references were taken of the rostral aspect of the larynges before and after abduction and the left arytenoid abduction distance was confirmed using image processing software (ImageJ, US National Institutes of Health, Bethesda, MD). Post-laryngoplasty abduction angle of the left arytenoid was also calculated from these photographs similarly to endoscopy photos of clinical cases after surgery. One ray of the angle was the midline sagittal plane of the larynx and the second ray was a line drawn from the proximal aspect of the corniculate process that extended ventrally on the medial aspect of the corniculate process of the arytenoid (Figure 4).

Figure 4 - Photograph of rostral larynx after laryngoplasty demonstrating left arytenoid abduction angle measurement
To standardize measurements all values were collected and recorded by the same investigator (NRM).

Larynges were embedded in 19 x 19 cm plastic containers half-filled with two part putty composed of polyester resin mixed with an organic peroxide hardener (Bondo® [3M Corp., St. Paul, MN]). Care was taken to make sure the larynx was firmly embedded in the Bondo, that the angle of abduction remained constant, and that the left arytenoid could move freely. When the polyester resin mixed with organic peroxide (Bondo®) was set, the postlaryngoplasty left arytenoid abduction distance was measured to determine if any loss of abduction had occurred during the embedding process. The larynges were oriented so that the vertical actuator of a servohydraulic test frame [Bionix 858, MTS Corp., Eden Prairie, MN] contacted the base of the spine of the left arytenoid muscular process where the spine blends into the body of the rostral aspect of the muscular process of the arytenoid perpendicular to its long axis (Figure 5).
Figure 5 – Illustration of left side of embedded larynx with actuator on base of arytenoid spine
The horizontal bar of the actuator was oriented parallel to the cricoid facet of the crico-arytenoid joint. The plastic container with Bondo was secured to the horizontal testing surface, and the larynx positioned so that when load was applied, the arytenoid rotated around the crico-arytenoid joint. A small amount (approximately a 7 mm round ball) of polymethylmethacrylate was placed on the base of the arytenoid spine and conformed to it and the part of the actuator that contacted the arytenoid to prevent slipping off target.

Each larynx was tested under cyclic loads of 2-26 N at a frequency of 1 cycle every 2 seconds (0.5 Hz) for 5000 cycles on a servohydraulic materials test frame (Bionix 858, MTS Corp., Eden Prairie, MN). Arytenoid displacement distance was recorded by the MTS software at the peak and valley of each loading cycle, and was analyzed in 1000 cycle increments.

Four larynges (2 clamped and 2 non-clamped) were imaged before left laryngoplasty and after testing using micro computed tomography (Siemens Preclinical Knoxville, TN) to characterize damage. Cross sectional imaging of the muscular process and dorsal cricoid cartilage in the transverse, sagittal and coronal planes were inspected using the Inveon Research Workplace software (Siemens Preclinical Knoxville, TN) to reveal cartilage injury associated with the suture. Three-dimensional reconstructions of suspected areas of cartilage tearing were performed to confirm damage. Laryngeal cartilage damage was determined to be present when irregular cartilage voids were present adjacent to embedded suture material. Three-dimensional overlay images were
also created to estimate shape change in the muscular process using cartilage mineralization as an internal control for position.

**Statistical Analysis**

Continuous data were analyzed using commercial statistical software [Microsoft Excel, Microsoft Corp., Redmond, WA and GraphPad Prism v5 GraphPad Software., La Jolla, CA]. Data for both clamped and non-clamped groups were assessed for normality using a Shapiro-Wilk test and found to be non-Gaussian in distribution. Comparisons between groups (clamped and non-clamped larynges) and between cycling intervals were assessed using a Mann-Whitney U test. Comparisons at different cycling intervals were assessed using a Friedman statistic for repeated measures with Dunn’s post hoc comparison testing for each group of larynges. Data are reported as median and range unless otherwise stipulated. Significance was set at P < 0.05.
Chapter 3: Results

There were seven larynges in each group (non-clamped and clamped). The median horse age was 3.5 years with a range of two to four years. The median age in the non-clamped group was 4 (range 3-4) years. The median age in the clamped group was 3 (range 2-4) years. There was no difference in the age of horse between groups, $P = 0.55$. Breeds represented included Thoroughbred (12) and one each of Standardbred and Quarterhorse.

*Non-clamped larynges*

The median left arytenoid length was 28.8 mm (range, 25.0 to 32.7 mm). The median post-laryngoplasty left arytenoid abduction distance for 7 non-clamped larynges was 17.1 mm (range, 9.8 to 19.8 mm), and the median left arytenoid abduction angle was 75 degrees (range, 60 to 87 degrees) (Table 2).
Table 2: Non-clamped Larynges

<table>
<thead>
<tr>
<th>Larynx</th>
<th>Left arytenoid length (mm)</th>
<th>Left arytenoid abduction distance (mm)</th>
<th>Left arytenoid abduction angle (Dixon)</th>
<th>Abduction loss after 1000 cycles (mm)</th>
<th>Percent abduction loss after 1000 cycles</th>
<th>Abduction loss after 5000 cycles (mm)</th>
<th>Percent abduction loss after 5000 cycles</th>
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<td>Early failure at 694 cycles</td>
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<td>Minimum</td>
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<td>Maximum</td>
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<td>0.02</td>
<td>0.17</td>
<td>0.01</td>
<td>0.13</td>
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</table>

* Mann Whitney U comparison between clamped and non-clamped larynges

Post cyclical testing left arytenoid abduction angle could not be determined due interference in angle assessment as a result of embedding. There were no changes in abduction distances following embedding of larynges. Five of 7 larynges completed cyclical testing for 5000 cycles and were included in statistical analysis of left arytenoid displacement. One larynx was stopped at 4000 cycles due to a computer error in the shut off criteria; it had a 43.5% loss of abduction at this point. Larynx number two was observed to be losing abduction rapidly at the start of testing so the 75% loss of abduction shut off point was deactivated to allow the specimen to reach 100% loss of abduction which occurred at 694 cycles. During the failure of this specimen, 2 mm of suture cut through was apparent at the caudal aspect of the cricoid cartilage and the caudal margin of the cricoid cartilage deformed rostrally during the testing. Due to the breach in our experimental protocol (see discussion) this specimen was also removed from further
statistical analysis. Median percent loss of left arytenoid abduction for five larynges after 5000 cycles in the non-clamped group was 31.9% (range, 22.4 to 64.0%).

**Clamped larynges**

The median left arytenoid length was 28.9 mm (range, 27.1 to 32.9 mm) (table 1). The median post-laryngoplasty left arytenoid abduction distance for 7 clamped larynges was 16.8 mm (range, 13.5 to 19.5 mm), and the median left arytenoid abduction angle was 70 degrees (range, 65 to 75 degrees). Six of 7 clamped larynges completed cyclical testing to 5000 cycles (Table 3).

**Table 3: Clamped Larynges**

<table>
<thead>
<tr>
<th>Larynx</th>
<th>Left arytenoid length (mm)</th>
<th>Left arytenoid abduction distance (mm)</th>
<th>Left arytenoid abduction angle (Dixon)</th>
<th>Abduction loss after 1000 cycles (mm)</th>
<th>Abduction loss after 1000 cycles</th>
<th>Abduction loss after 5000 cycles (mm)</th>
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<td>Maximum</td>
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<td>75</td>
<td>7.8</td>
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</tr>
</tbody>
</table>

P-value: 0.08 0.04 0.32 0.02 0.17 0.01 0.13

*# Mann Whitney U comparison between clamped and non-clamped larynges*

Similar to the non-clamped group, one larynx demonstrated a rapid initial loss of abduction that was allowed to continue until 100% loss occurred after 58 cycles. This
specimen had 3mm of suture cut through at the caudal aspect of the cricoid and also displayed rostral deformation of the cricoid during the cyclical testing. Due to the breach in protocol, the larynx was removed from further statistical analysis. The median percent loss of left arytenoid abduction for six larynges after 5000 cycles in the clamped group was 46.2% (range, 33.2 to 75%).

There was no difference between the clamped and non-clamped larynges in median left arytenoid length (P = 0.68), left arytenoid abduction distance (P = 0.84) or left arytenoid abduction angle (P = 0.32). Clamped larynges showed more absolute arytenoid abduction loss as compared to non-clamped larynges at 5000 cycles (p=0.01), but there was no difference in the percentage loss of abduction between groups at 5000 cycles (p=0.13). The percentage loss of abduction was also analyzed for subsets of 0-1000 cycles, 1001-2000, 2001-3000, 3001-4000, and 4001-5000. No difference in the percent abduction loss was observed for cycle subsets between clamped and non-clamped larynges (1000 cycles, P = 0.17; 5000 cycles, P = 0.13), however the median percent loss for the first 1000 cycles was significantly greater in both clamped and non-clamped larynges than any other test cycle sub-set (median of 37.6% and 28.8%; P<0.01).

Mode of failure

The two larynges that failed early in testing had dynamic rostral deformation and tearing of the caudal border of the cricoid cartilage by the suture. Tearing injury or compression of the cartilage was apparent at three locations in the four larynges imaged with computed tomography: the rostral suture puncture of the cricoid (3 larynges), the caudal edge of the cricoid (3), and the rostral suture puncture of the muscular process (3).
Three larynges had evidence of cartilage damage at more than one location, and one had only severe tearing of the rostral suture puncture of the cricoid cartilage (Figure 6a). One larynx had cartilage tearing at the rostral abaxial aspect of the muscular process with a 4 mm long irregular transverse fracture line (Figure 6b).
Figure 6 – a: Reconstructed micro-CT image from left dorsal perspective of post-test larynx from clamped horse 1. Note severe damage to cartilage at rostral suture puncture (arrow) in cricoid cartilage, and an undamaged muscular process. b: Reconstructed micro-CT image of lateral aspect of fractured muscular process of the arytenoid after testing from non-clamped horse 4. There is an irregular partial-thickness transverse crack in the muscular process at the rostral aspect of the suture puncture.
One larynx did not have mineralization in the muscular process, so it could not be registered for shape change. However, the three remaining larynges had evidence of subtle plastic deformation of the muscular process after suture placement and cyclical testing, specifically, a depression on the rostral surface of the arytenoid spine corresponding to the level of the suture bite in the muscular process and caudal stretching of the caudal aspect.
Chapter 4: Discussion

The ideal amount of postoperative abduction of the left arytenoid after laryngoplasty for the racehorse is unknown. Computer modeling has shown that surgical arytenoid abduction that achieves 88% of the maximal cross-sectional area of the rima glottidis is most appropriate, and that lesser abduction reduces airflow, the latter of which was confirmed by a cadaver study. However, clinical investigations have not been able to differentiate between mild and moderate degrees of postoperative abduction and racing performance. Poor outcomes were associated with excessive or inadequate abduction of the left arytenoid cartilage, but statistical significance was difficult to achieve due to relatively low numbers of horses in these groups.

Adductory forces on the abducted arytenoid cartilage occur cyclically during swallowing and coughing. The cricopharyngeus muscle functions as the rostral esophageal sphincter in the dogs and humans, and we hypothesize functions similarly in the horse. It has been speculated that contraction of the thyropharyngeus and cricopharyngeus during swallowing directs a force to the spine of the muscular process causing the arytenoid cartilage to adduct. This model was developed to mimic this adductory force to investigate its effect on post-laryngoplasty abduction when laryngoplasty is performed after abduction by suture alone or when performed aided by a purpose-designed clamp.
A recent in vivo study showed that the laryngoplasty suture is subjected to an increase in force over baseline of 19.0 ± 5.6 N and 12.1 ± 3.6 N respectively, during swallowing and coughing experimentally induced by instilling a small volume of saline into the nasopharynx or trachea\textsuperscript{104}. These forces occurred a mean of 1152 times in 24 hours\textsuperscript{104}. However, spontaneous suture loading during activity in the stall resulted in a larger force of 26.9 ± 6.9N, and one horse had forces of 41.8N\textsuperscript{104}. These spontaneous forces occurred at a mean frequency of 908 cycles with a range of 429 – 1376 times in a 24 hour period. We used a cyclic force of 26N to approximate the forces an abducted left arytenoid would experience in the immediate postoperative period. We chose to test our larynges non-destructively to 5000 cycles at (0.5Hz) to provide several days worth of force in a short period of time (≈ 3 hour total test time) to reduce desiccation of our specimens.

To measure left arytenoid abduction distance, we modified a previously reported method\textsuperscript{85} because the quantitative output from the Instron indicated left arytenoid displacement from the baseline abducted position, and using a similar measurement to describe the abduction distance would allow calculation of the percent change. The procedure had the advantage of requiring very little equipment to perform, took advantage of consistent easily defined anatomical structures, was inexpensive, and did not require a lot of pretest set time. We also measured the resultant angle of left arytenoid abduction from photographs using NIH image using a method similar to a clinical description\textsuperscript{92}. In all 14 larynges, a Dixon grade 2 abduction (50-80°) was achieved.
In both groups a significant percent of post-laryngoplasty abduction loss occurred within the first 1000 cycles in comparison to the loss of abduction at all other cyclic time intervals (P < 0.01). For all larynges, the median percent loss of abduction during the first 1000 cycles was 35.4%. This is a similar degree of arytenoid abduction loss as is seen in the first week after surgery. The greater loss of abduction during the first 1000 cycles is probably due to the laryngeal cartilage and other soft tissues under the suture accommodating to the suture force. Another explanation for the major losses of abduction that were observed in this model after approximately the number of cycles that a larynx would experience in a day could be the high frequency rate of cycling used. Cycling at a rate of 0.5 Hz would clinically mean a larynx experiences an adductory force (cough or swallow) every 2 seconds which is unlikely to occur after surgery.

During testing of one larynx in the non-clamped group, the computer stopped at 4000 cycles due to a technical error. Despite only running 4000 cycles, this larynx still experienced a 43.5% loss of abduction that occurred mostly in the first 1000 cycles. The mode of failure appeared to be cut through at the level of the cricoid cartilage based on gross evidence of a 3 mm groove in the caudal cricoid cartilage. Additionally, one larynx in each group failed early in testing (58 and 694 cycles). Both of these larynges displayed a noticeable rostral deviation of the cricoid cartilage and a small amount of caudal cricoid suture pull through during cyclic loading. The reason for early failure is not known, but could be the result of variation in the material properties of individual larynges. At surgery, it is common for there to be a variation in the pressure required to penetrate laryngeal cartilage with the needle. Laryngeal cartilage material properties also change
with age and differ by location within a cartilage\textsuperscript{111}. The dynamic displacement of the caudal border of the cricoid seen in the larynges failing early in testing could be the result of a lack of soft tissue attachments to the cricoid and the absence of esophageal pressure on the dorsal cricoid surface, or reduced stiffness of the caudal aspect of the cricoid\textsuperscript{111}.

Unlike high load single cycle to failure models that fail at the suture or tear grossly through the cartilage, failure in tested larynges that completed the 5000 cycles was less obvious, and possibly better represents in vivo failure. Our cycling model indicated several possible locations that could contribute to laryngoplasty failure, and more than one mode could be seen in an individual larynx. Failure modes included dynamic rostral deformation of the cricoid, cutting through of the cricoid by the suture either at the caudal aspect or at the rostral needle puncture, and small fractures of the muscular process. Changes in cartilage shape after testing was difficult to quantify due to the change in position of the cricoid and arytenoid cartilages after laryngoplasty; however mineralization in the muscular process allowed for an overlay of pre-suture and post-testing images for three larynges. In these specimens, the muscular process appeared to be slightly deformed after testing in the area of the suture bite. Future testing should use standardized reference controls on the larynx to estimate plastic deformation.

Our hypothesis that abducting the arytenoid with a clamp to reduce cartilage tearing by the suture during abduction would improve the maintenance of prosthetic arytenoid abduction when subjected to cyclical adductory forces was not supported. Although the median percent loss of arytenoid abduction after 5000 cycles was not statistically significant (P = 0.13) between groups, the clamped group had a trend towards
a greater degree of median percent loss of abduction (46.2%) compared to the non-clamped group (31.9%). This may be the result of initial cartilage compression or tearing when clamped larynges are first challenged by the suture. Using the suture in the non-clamped group to create a firm connection before knot tying may result in less total arytenoid displacement after challenge. This result supports the technique of “setting” the suture in cartilage by small sawing motions before knot tying (D.E. Slone, personal communication, 1986).

Any ex vivo model has limitations in replicating the clinical situation, and this model is not different. Isolating the larynx from the surrounding soft tissues will remove some normal forces on the larynx and possibly influence cartilage behavior when subjected to adduction; this may explain the rostral motion of the caudal aspect of the cricoid in the larynges that failed early in testing. Freezing and thawing of the larynges could alter the material properties of the cartilage, although recent testing indicates that is not the case. To ensure even force on the cartilage, only one suture was used to abduct the arytenoid, and most surgeons would use two in a patient, and that could alter the mode of failure. To prevent desiccation of the larynges, the frequency of cycling was fast compared to swallowing in a clinical patient, and that could have accelerated the early loss of abduction. However, our model approximates the cyclical forces that are placed on left arytenoid cartilages abducted via laryngoplasty. These results combined with the loss of abduction seen in clinical cases provide strong evidence that cyclical adductory forces contribute to early postoperative loss of laryngoplasty abduction. We believe that this model can provide experimental data that could be useful to understand laryngoplasty
failure in the early postoperative period, and to develop strategies to improve the maintenance of left arytenoid abduction in horses after laryngoplasty.
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