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DYNAMIC EQUINE LARYNGEAL PROSTHESIS

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ABSTRACT

The respiratory system of horses is the major limiting factor for athletic performance. As such, any respiratory impairment can have a considerable effect on athletic performance. A common site for this impairment to occur is the upper respiratory tract with laryngeal collapse being the most common form in horses. The recurrent laryngeal nerves in the horse are the longest nerves in the body and prone to degenerative axonopathy. This is termed recurrent laryngeal neuropathy (RLN) and results in neurogenic atrophy of the cricoarytenoideus dorsalis (CAD) muscle. Loss of function of the CAD leads to dynamic laryngeal collapse when exposed to the negative airway pressures produced during exercise. As a result, RLN is a common cause of reduced athletic performance in horses. Currently the most commonly performed treatment for RLN in horses is a static prosthetic laryngoplasty. This procedure has a relatively poor success rate in performance horses (ranging from 50-70%) and high complication rate of between 26-43%.[1-4] Two of the major complications are dysphagia and loss of abduction. In general, many of the complications can be attributed to either under or over abduction of the arytenoid and static laryngoplastic fixation.

The objective of the research reported here was to develop a laryngoplasty system that allowed for alteration of the degree of arytenoid abduction post-operatively. This thesis outlines the successful development of such a device.

Initially, the existing standard laryngoplasty procedure was examined mechanically and a variety of prototypes were developed and evaluated. Subsequently, we selected the final dynamic laryngoplasty system (DLPS) for further evaluation. After optimising the position of attachment to the arytenoid using an anchor, the prototype device was mechanically tested under static loading, cyclic and ramp (single linear loading) to failure conditions. This study found the device was able to cause effective shortening of a suture loop with

minimal cyclical loss and was able to resist ramp testing sufficiently to justify further evaluation. Additional *in vitro* testing was performed using cadaveric larynges and demonstrated that the DLPS was able to cause effective increases in arytenoid abduction within the confines of equine laryngeal anatomy. The final *in vitro* study tested the ability of the DLPS to achieve and maintain arytenoid abduction during testing using a static airflow model with a flow rate of 55L/sec which was consistent with previously published research. After completion of the *in vitro* studies an *in vivo* proof of concept study was performed. This demonstrated that the device could be effectively delivered via a standing procedure under sedation and allowed for selective alteration in arytenoid abduction at 7 days post-operatively.

DECLARATION BY AUTHOR

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Peer-reviewed papers

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KEYWORDS

Equine, larynx, laryngoplasty, dynamic laryngoplasty system, DLPS, tie-back, recurrent laryngeal neuropathy (RLN)

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PART I: GENERAL INTRODUCTION

Chapter 1: Introduction, literature review and project aims

1.1 Introduction

The major limiting factor in a horses' exercising capacity is the respiratory system.[5] Within this system the upper airway is a common site of obstruction to airflow that can result in poor performance. The importance of the respiratory system in horses is illustrated by the massive increase in airflow associated with exercise. At rest horses have a tidal volume of approximately 5 L and breathing rate of 15 breaths per minute leading to a minute ventilation of 75 L per minute. With exercise this increases by between 20-60 times to 1200-2580 L per minute.[6-10] These massive airflow rates lead to large negative airway pressures affecting the upper airway.[6,11] Peak negative airway pressure during inhalation has been reported to range from 3922 to 4903 Pa whilst galloping at 14m/sec on a treadmill with a peak inspiratory flow rate of 2580L/min.[9,12] Humans in comparison have a negative pressure of only 441 Pa during a forceful sniff with a flow rate of 1.1L/sec.[12,13] Horses are obligate nasal breathers. This means they are unable to breathe through the oral cavity and all air has to pass through the nares. As a result, horses must accommodate the massive increases in airflow of exercise solely through the nasal cavity. During inspiration 90% of the resistance to airflow is attributed to the upper airway and this is evenly distributed between the nares and the larynx.[6,12] The nares are rarely a cause of clinically relevant increases in airway resistance in horses. However, in contrast, the larynx is an important site of increased airflow resistance even in a normal functioning state. When the larynx is dysfunctional it can cause a massive negative effect on airflow and performance as a result.

The larynx is short tube that connects the pharynx to the trachea.[14] It is an anatomical

structure that is uniquely required to serve two major functions: facilitating airflow during breathing; and preventing water and food from entering the trachea during deglutition. To achieve its required functions the larynx needs to be able to close fully during swallowing and then open maximally during exercise. Abduction of the arytenoid cartilages results in enlargement of the *rima glottidis* and is controlled in the horse by a single pair of muscles called the *cricoarytenoideus dorsalis* (CAD). These muscles, one on each side of the larynx, are solely responsible for opening each hemi-larynx to a point that they minimally obstruct airflow. These muscles are innervated by the left and recurrent laryngeal nerves which are branches of the vagus. The left recurrent laryngeal nerve is the longest nerve in the horse's body measuring up to 250 cm in length, twice as long as other motor nerves in the horse and approximately 31 cm longer than the right side.[15] The greater length on the left is due to the nerve coursing around the aortic arch, compared with the right which does not. As a result of this unique anatomy the left recurrent laryngeal nerve has historically been thought to be most susceptible to degeneration. However, the role of nerve length in the pathogenesis of this degeneration is uncertain at this stage.[16] Degeneration of the recurrent laryngeal nerve is commonly termed recurrent laryngeal neuropathy (RLN). This nerve dysfunction then leads to CAD muscle atrophy and hence an inability to sufficiently abduct the affected left arytenoid cartilage.[17-20] When complete, this has been termed laryngeal hemiplegia (LH). This reduction in the ability to open the larynx causes increased resistance to airflow and results in reduced oxygen delivery leading to reduced athletic performance. [21,22]

Recurrent laryngeal neuropathy, resulting in laryngeal collapse has been recognised as a performance-limiting disease process in horses since the 1800s.[23] The neuropathy is likely to have a genetic basis however the precise aetiology remains uncertain.[18,24-26] It is caused by a progressive distal axonopathy and has historically been thought to affect multiple

nerves in the body but with the left recurrent laryngeal nerve most severely affected. However, recent histological evaluation of the recurrent laryngeal nerve and other nerves has determined the degeneration to be more correctly termed a bilateral mononeuropathy with the degeneration limited to the recurrent laryngeal nerve.[27] The prevalence of RLN varies between breeds with larger breeds more commonly affected.[28,29] In Thoroughbreds the prevalence of RLN has been reported based on resting endoscopic findings to be between 2.6-8%.[30] In heavy draught horse breeds a prevalence of up to 35% has been reported.[31]

1.2 Equine Laryngeal Anatomy

The larynx in the horse consists of three single cartilages (cricoid, thyroid and epiglottic) and the paired arytenoid cartilages (Figure 1). [14,32]

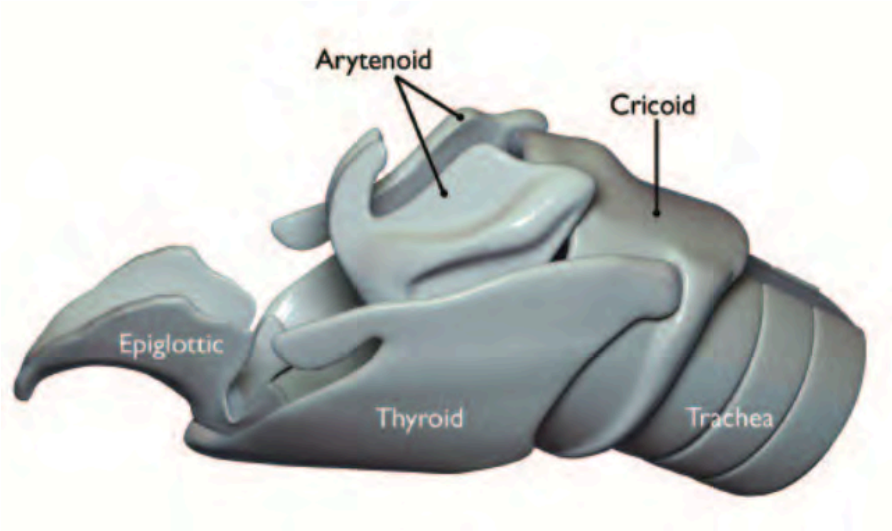

Figure 1. Single cricoid, thyroid, and epiglottic cartilages and paired arytenoid cartilages form a communicating channel between the pharynx and trachea. Adapted from Janicek et al. 2008.

The cricoid and thyroid cartilage are both composed of hyaline cartilage with the epiglottis being elastic cartilage. The arytenoids are a combination of cartilage types with the muscular process being hyaline with the remainder being elastic. The cricoid cartilage is signet ring shaped and articulates with the thyroid at the cricothyroid articulation. It also articulates with each of the arytenoids at the cricoarytenoid joint which is a diarthrodial saddle-shaped joint. This joint is located such that the arytenoids are situated on either side and in front of the cricoid and partly medial to the thyroid cartilage. The arytenoids are slightly pyramidal in form and have three processes: the muscular, the vocal and the corniculate process.[14] The cricoid cartilage has been found on computed tomographic imaging to have notable conformational differences at the caudal edge in Thoroughbred larynges.[33] These differences are also markedly evident during dissections of the equine larynx. The CAD muscle is a strong fan shaped muscle that attaches to the apex of the muscular process of the arytenoid. A cartilaginous ridge extends cranially from the apex of the muscular process along a line parallel to the orientation of the CAD muscle and is termed the arcuate crest (*crista arcuata*). The paired left and right CAD cover the dorsal surface of the cricoid and each has two neuromuscular components.[34] Each muscle originates from half of the caudal cricoid lamina including the median ridge and the fibres converge into a tendon that inserts onto the muscular process of the arytenoid.[14] The action of the CAD muscle is to dilate the *rima glottidis* by rotating the arytenoid at the cricoarytenoid joint into an abducted position. The recurrent laryngeal nerve innervates the CAD muscle and this is the only abductor of the larynx. The recurrent laryngeal nerve also innervates the cricoarytenoideus lateralis (CAL), the arytenoideus transversus and the thyroarytenoideus muscle group (combined ventricularis and the vocalis muscles) (Figure 2).[14,16] All of these muscles are associated with adduction of the larynx and are similarly affected to the CAD when there is RLN.[35]

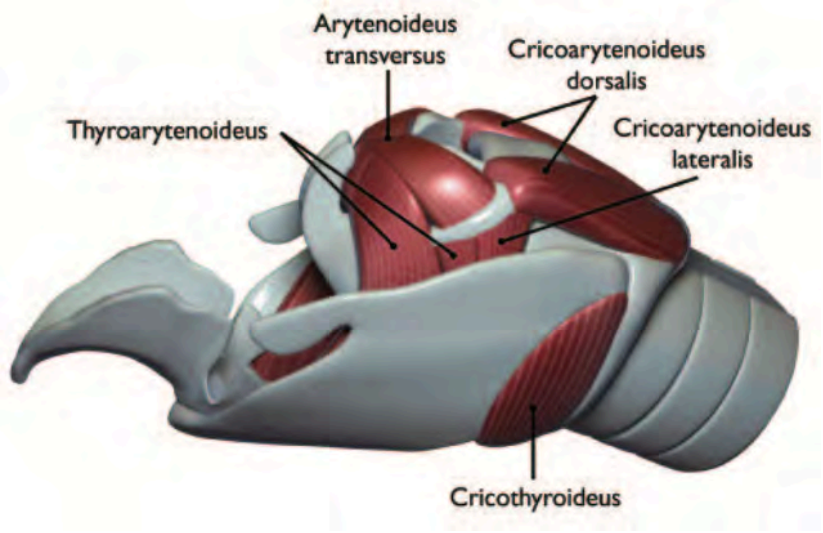

Figure 2. Overlay of intrinsic laryngeal muscles that abduct and adduct the diameter of the rima glottidis via contraction. Adapted from Janicek et al. 2008.

1.3 Respiratory physiology related to laryngeal dysfunction

The respiratory system is the limiting factor for athletic performance in horses. [6,36,37] This is due to the delivery of oxygen being the primary limiting factor to increases in VO_{2max} during strenuous exercise.[38] The equine cardiovascular and muscular systems can transport and utilise more oxygen than the lungs can supply.[36] As a result during strenuous exercise normal horses develop hypoxaemia (<60 mmHg) that will negatively impact further aerobic performance.[38] Any impairment of the equine respiratory system such as laryngeal collapse caused by RLN, will compromise athletic performance. This has been demonstrated clearly in exercising horses where transection of the recurrent laryngeal nerve significantly increased respiratory impedance and worsened exercise-induced hypoxemia. [10,19] Horses with RLN cannot achieve maximal abduction of the affected arytenoid cartilage, and as negative inspiratory pressure increases with exercise, leads to progressive narrowing of the *rima glottidis* due to arytenoid collapse. This subsequently

leads to hypoxemia, hypercarbia and metabolic acidosis occurring more quickly in horses affected with RLN compared to non-affected horses and results in musculoskeletal fatigue and poor performance.[22,39] However, research has shown that the currently performed LP improves airflow and reduces inspiratory resistance but does not restore airway mechanics or arterial blood gases to normal limits.[10,19,22,40] Furthermore, one study found that the performed LP diminished normal laryngeal protective mechanisms leading to airway contamination.[10]

1.4 Aetiology and pathophysiology of RLN

Laryngeal dysfunction is a common equine clinical problem and a genetic basis is most likely, however, in a majority of affected horses the precise aetiology is uncertain.[41] As a result, between 89-94% of cases of laryngeal hemiplegia of unknown aetiology are attributed to idiopathic RLN.[26,42] The cause of RLN been hypothesised to range from mechanical stretching of the recurrent laryngeal nerve during neck movement, growth and caudal movement of the heart during embryogenesis to environmental factors, including toxins.[16] Histologically, idiopathic RLN is characterised by distal degeneration of the recurrent laryngeal nerve.[27,35,43] However, other disorders can cause damage to the recurrent laryngeal nerve and this has been previously reported to be the case for the remaining 6-11% of horses presented with LH. [26,42] These include guttural pouch mycosis, perivascular injections of irritant drugs in the area of the recurrent laryngeal nerve, neck trauma and neoplasia.[26] Of horses with a specifically identified cause of RLN more than half of these cases were bilaterally affected. [26,42] Regardless of the exact aetiology, the result is neurogenic atrophy of the laryngeal musculature, which mechanically results in an inability to effectively abduct the affected arytenoid.

1.5 Diagnosis of RLN

Recurrent laryngeal neuropathy results in neurogenic atrophy of the CAD and leads to an inability to abduct the affected arytenoid effectively from the airway. The diagnosis of RLN can be suspected on the basis of history and physical examination.[44] However, as previously discussed, RLN and the subsequent CAD atrophy varies in severity. As a result, there are differing effects on performance depending on the degree of airway obstruction and the nature of the intended athletic function of the horse. This results in the disorder having a range of effects on performance from career limiting to clinically insignificant inspiratory noise. As such, advanced diagnostic measures have been developed to accurately diagnose and classify all affected horses in an effort to target treatments more effectively. Diagnostic tools that have been investigated in the evaluation of RLN in horses include: standing endoscopy, dynamic endoscopy, laryngeal ultrasound, exercise spirometry, sound analysis, electromyography, CT and MRI.[19,45-50] The current gold standard for diagnosis of RLN is considered to be an exercising (dynamic) endoscopic examination, which may be performed either on a high-speed treadmill or "over ground".[5] The video-endoscopic images are recorded and can be played back frame by frame to allow for accurate diagnosis and classification of the type and severity of upper airway dysfunction present in any horse suspected to be suffering from RLN.[47,51-53]

Several grading systems have been developed for standing endoscopic assessment of laryngeal function.[54,55] These grading systems categorise horses based on arytenoid abduction function at rest in an effort to grade the severity of dysfunction and draw parallels relating to the likely corresponding function during exercise. As a result, standing endoscopic grading was developed to allow for consistency in reporting of this condition and also to allow evaluation of treatment success. A seven-grade system has been

158 developed and is widely accepted for use during standing endoscopic examinations as
159 proposed initially by the Havemeyer workshop group and international experts in 2003
160 (table 1).[55]

Grade	Description	Sub-grade
1	All arytenoid cartilage movements are synchronous and symmetrical and full arytenoid cartilage abduction can be achieved and maintained.	
2	Arytenoid cartilage movements are asynchronous and/or larynx is asymmetric at times but full arytenoid cartilage abduction can be achieved and maintained.	2.1 Transient asynchrony, flutter, or delayed movements are seen. 2.2 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.
3	Arytenoid cartilage movements are asynchronous and/or asymmetric. Full arytenoid cartilage abduction cannot be achieved and maintained.	3.1 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. 3.2 Obvious arytenoid abductor deficit and arytenoid asymmetry. Full abduction is never achieved. 3.3 Marked but not total arytenoid abductor deficit and asymmetry with little arytenoid movement. Full abduction is never achieved
4	Complete immobility of the arytenoid cartilage and vocal fold.	

161 Table 1. Havemeyer grading system of laryngeal function in the standing unsedated horse.
162 This grading generally refers to the left arytenoid cartilage in reference to the right but can
163 be applied to right sided dysfunction.

164

In Australia and New Zealand a five-grade scale (table 2) has been used historically but is gradually being replaced with the aforementioned Havemeyer scale.[54]

Grade	Description
1	All movements, both adduction and abduction are **synchronised and symmetrical**.
2	All major movements are symmetrical and a full range is achieved. **Transient asynchrony**, flutter or delayed opening may be seen.
3	Asymmetry of the rima glottidis at rest due to reduced motility by the left arytenoid cartilage and vocal fold. On occasions, typically after swallowing or during the nostril closure manoeuvre, **full symmetrical abduction is achieved**.
4	There is consistent asymmetry of the rima glottidis but with some residual active motility by the left arytenoid cartilage and vocal fold. **Full abduction is not achieved at any stage.**
5	True hemiplegia. There is obvious and consistent asymmetry of the rima glottidis with **no residual active motility** by the left arytenoid cartilage and vocal fold.

Table 2. Five-point grading system as per Lane 2004.

The use of these resting endoscopic grading systems have been shown to be subjective and influenced by factors such as sedation, which nostril the examination is performed via and the day of the examination.[56]Further studies have shown that the correlation between resting grades and dynamic laryngeal collapse is not highly reliable.[47,52,57,58] Laryngeal ultrasound has been shown to have a higher sensitivity and specificity (90% and 98% respectively) than resting upper airway endoscopy (80 and 81% respectively) in the prediction of abnormal arytenoid movement determined during a treadmill upper airway endoscopic examination.[59]

As a result of the inaccuracy of using standing laryngeal grade as a predictor of laryngeal

collapse methods of performing dynamic examination of the upper respiratory tract have been developed and are considered the gold standard diagnostic technique. These examinations are commonly performed using a high-speed treadmill or more recently using an over ground endoscopy system. A grading system to use during these dynamically exercising examinations has been proposed by Rakestraw (table 3).[60] This system differentiates based on the degree of arytenoid collapse at exercise.

Grade	Description
A	Full abduction of the arytenoid during inspiration
B	Partial abduction of the affected arytenoid cartilage (between resting position and full abduction)
C	Abduction less than resting position, including collapse into the contralateral half of the rima glottidis during inspiration

Table 3. Grading system of laryngeal function as assessed during exercise. This grading generally refers to the left arytenoid cartilage in reference to the right but can be applied to right sided dysfunction.

1.6 Treatment options for RLN

Several treatment options are available for horses with RLN and which option is selected is often based on the nature of the presenting complaint, the age of, and the intended future use of the horse. For horses required to perform at maximal levels of performance such as racehorses, the surgical treatments available include a prosthetic laryngoplasty, ventriculectomy, ventriculocordectomy, re-innervation of the CAD muscle and partial arytenoidectomy. The most commonly selected procedure in horses that are required to work and race at maximal exercise capacity for distances greater than 800m is a prosthetic laryngoplasty.[61] This is commonly combined with either a ventriculectomy or a

ventriculocordectomy which may be performed unilaterally or bilaterally to abolish vocal fold collapse and reduce respiratory noise.[1,4,62,63] Where noise is the predominant complaint, such as in performance horses, then a ventriculectomy or a ventriculocordectomy alone can be effective in reducing respiratory noise.[40,62,63] Neuromuscular pedicle grafts can be used to re-innervate the CAD muscle and are suitable for any affected horse but used most commonly in young horses with grade 3 laryngeal movement, where return to athletic function is not expected within 4 months of surgery.[20] Successful re-innervation of the CAD following neuromuscular pedicle grafts can take 6-12 months and is not, as a result, always suitable for horses that are required to return to athletic performance quickly. A partial arytenoidectomy is often used as the treatment option for failed laryngoplasty surgeries or in cases of arytenoid chondritis causing malformation of the arytenoid cartilage. This has been found to improve airflow dynamics compared to an induced laryngeal hemiplegic state but not back to normal values and not as significantly as a laryngoplasty.[10]

1.7 Current Laryngoplasty Techniques, Success Rates and Complications

1.7.1 Laryngoplasty technique

Treatment by laryngoplasty was first reported by Cadiot in 1893, who transcutaneously sutured the paralysed arytenoid to the thyroid cartilage.[64] With remarkably little variation, the technique described by Marks and colleagues in 1970 is still accepted as the treatment of choice, despite the limited success and host of possible complications.[65] This technique fundamentally places a prosthesis to replace the function of the atrophied CAD muscle. Generally speaking, a suture material is placed from the cricoid to the muscular process of the arytenoid and then tightened to achieve the desired degree of arytenoid abduction. The

arytenoid is then fixed in a static position.

1.7.2 Success Rates

The reported success rate of the modern laryngoplasty procedure is extremely varied and ranges from 5% to 95%.[1,2,66-68] Of interest, there has not been a marked improvement in reported success rates over time.[1,61,66,69,70] The remarkable variation in reported success rates is due to the different criteria used to measure success and the intended use of the horse. Thoroughbred racehorses have a lower success rate (48-66%) compared to breeds not intended for racing (90-95%).[1,2,67,69] Laryngoplasty in horses not intended to race, or to race over short distances (<800m) will be more successful due to the lower intensity of performance or reduced reliance on aerobic metabolism respectively required post-surgery.[61]

The current prosthetic laryngoplasty is a commonly performed procedure that has significant scope for improvement. Clearly, the success rate is strongly dependent on the intended use of the horse but for maximal athletic performance the success rates are relatively poor.[1,2,61,67,70] Because of this scope for improvement an enormous number of variations on the basic theme of a static laryngoplasty have been developed and subsequently researched with none of them being uniformly embraced by the equine surgical community.[4,71-75]

1.7.3 Complications

Due to the static nature of the laryngoplasty procedure, the horse's larynx is permanently affixed in a partially abducted position post-operatively. This position is a compromise between the requirements of the larynx to be abducted for maximal airflow yet still being able to function in preventing food contamination of the airway. The complications

246 associated with the laryngoplasty procedure are mostly caused as a result of this
247 compromise. Many of these complications can be related to either over or under abduction
248 of the statically abducted arytenoid.

249 A variety of complications have been attributed to over abduction and dysphagia as a result
250 although this is not always a direct linear relationship.[61,68] Post-operatively, 9-16% of
251 horses that underwent a laryngoplasty procedure have been reported to have some degree
252 of nasal discharge in the immediate postoperative period.[1,3] Coughing has been reported to
253 occur in 13% of draft breeds, in 18-33% of mixed breeds, and in 26-43% of racehorses
254 undergoing laryngoplasty.[1,2,4,66,76,77] In some cases, coughing may solely be a manifestation
255 of over abduction of the arytenoid.[78] Given that the most common cause for post-operative
256 coughing is aspiration of feed material, most management procedures are aimed at
257 reducing this complication by reducing aspiration. Although marked dysphagia was
258 associated exclusively with high amounts of abduction in one article, other investigators do
259 not believe this association has a direct linear correlation.[61,68] Rates of intraoperative target
260 abduction vary depending on the study and have been reported to be 50-90%.[4,56,68]
261 Furthermore, one study reported that abduction above resting position did not improve
262 performance but increased the rate of complications.[2] It is commonly accepted that
263 laryngoplasty procedures result in airway contamination over normal or hemiplegic
264 states.[10] When excessive abduction is observed post-operatively and combined with
265 significant coughing and dysphagia, a second surgery to remove, replace, or loosen the
266 prosthesis is often recommended.

267 Complications due to loss of, or under, abduction are mostly related to poor exercise
268 performance. In general, loss of abduction is a well-recognized phenomenon of the
269 currently performed static laryngoplasty procedure and occurs most commonly in the first
270 7 days.[66,68] Although greater abduction has been associated with higher rates of

271 complications, it has also been associated with a greater likelihood to return to work and
272 improved performance.[2,77] As a result, the optimal degree of abduction is difficult to
273 evaluate at the time of surgery and even more difficult to maintain post-operatively.
274 Furthermore the “ideal” degree of abduction is likely highly variable and will likely need
275 to be determined on a case-by-case basis. For example, for horses performing at
276 submaximal levels (e.g., jumpers, draft horses), the concern is more often with reduction in
277 noise and not necessarily maximal airflow. Hence, a laryngoplasty at a lower percentage of
278 maximal abduction may be sufficient for their purpose and may not have as many
279 postoperative complications. In contrast, racehorses require maximal airway dilation to
280 allow them to compete and, as a result, may require more aggressively abducted
281 laryngoplasty procedures but with a static procedure these will inevitably be associated
282 with much higher rates and severities of complications. Satisfactory abduction at 9 days
283 postoperatively was achieved in only 77% of horses undergoing laryngoplasty surgery,
284 with failures mostly attributable to suture cutting through the laryngeal cartilages.[66] These
285 failures occurred in two 2-year-olds and a 3-year-old, leading the authors to hypothesise
286 that the cartilage of young horses was weaker and more prone to failure compared with
287 older horses.[61] This claim was refuted, however, in an *in vitro* study determining pull-
288 through forces that indicated no difference related to age.[79] Furthermore, the density of the
289 cricoid cartilage has been found to be unrelated to age, suggesting that laryngoplasty
290 failure is independent of this factor.[79] Of 200 laryngoplasty surgeries performed in one
291 study, 5% had repeated surgery to tighten or replace prostheses within 7 days
292 postoperatively.[68] In addition to this short-term loss of abduction, longer-term loss of
293 abduction is also a commonly reported. It is often extremely difficult to determine the
294 exact reason for this failure. Complications such as prosthesis or cartilage failure are two
295 commonly postulated reasons. Attempting efforts to determine the reason for these long-

term failures. Suggested factors include acute mechanical cartilage failure, cyclic cartilage failure resulting in gradual prosthesis loosening, improper prosthesis placement resulting in biomechanical disadvantage and any disease state rendering cartilage weaker than normal.[77] Efforts to reduce acute mechanical pull-out using novel prosthesis systems have been performed but have not gained widespread acceptance.[75,80] Recurrent laryngeal neurectomy to abolish any residual adductor muscle activity that may contribute to continued cyclic forces acting on the prosthesis was not effective in improving postoperative racing performance.[77] Improper placement of the prosthesis overly lateral on the cricoid results in loss of mechanical advantage and can lead to decreased abduction.[33]

Horses that have undergone a laryngoplasty have been shown to be at an increased risk of suffering from exercise induced pulmonary haemorrhage post-operatively.[81]
Laryngoplasty failure due to persistence of an abnormal respiratory noise or poor performance can be due to laryngoplasty failure in general or due to the development of other forms of dynamic laryngeal collapse such as dorsal displacement of the soft palate.[82]

1.8 Biomechanics of Laryngoplasty

The current traditional prosthetic laryngoplasty utilises a prosthetic material to replace the compromised CAD muscle function of the larynx and so achieve arytenoid abduction, allowing for effective airflow. However, whilst the basic premise of the laryngoplasty has not changed, the surgical techniques currently employed to achieve this are extremely varied (e.g. suture type, suture numbers, position) and have been associated with unreliable

results. These static surgical options focus on the abduction of the arytenoid to a predetermined position and maintaining it in that position.

There are four central components to the laryngoplasty construct. These are; the arytenoid cartilage, 2) the cricoid cartilage, 3) the cricoarytenoid articulation and 4) a prosthetic suture/material. How these segments interact determines the effectiveness of obtaining and maintaining effective arytenoid abduction. Recent research has focused on the interplay of these central components and attempted to maximise the stability of the surgical construct. Mechanical research of the laryngoplasty has historically focused on single cycle testing to failure.[79,80,83-85] Recently, cyclical models of laryngoplasty testing have been reported and are likely a more critical evaluation of traditional and novel laryngoplasty techniques as they test smaller repeated forces on the construct which is more comparable to the stresses on the construct during breathing, coughing and swallowing.[75,86]

The load applied to the cartilage-suture construct *in vivo* is an important factor required for effective mechanical evaluation of a laryngoplasty construct. An *in vivo* study evaluated the force required to achieve optimal arytenoid abduction, which was found to be 27.6±7.5N. During swallowing and coughing, the mean force on the suture increased by 19.0±5.6N and 12.1±3.6N, respectively.[87,88] Additionally, these horses were instrumented for 24hrs and it was found that the suture underwent cyclical loading due to swallowing at a mean of 1152 times in that period, a rate of 0.8 times per minute. From this research, it was estimated that the peak force on a laryngoplasty construct would be 46.6N. This reported force to obtain abduction was much higher than the force reported to cause maximal abduction in a previous study that was only 14.7N.[89] However, this was an *in vitro* study on cadaveric larynges and the load may have been lower than *in vivo* due to the absence of muscular function or different suture placement positioning.

1.8.1 The arytenoid cartilage

The arytenoid cartilage has historically been suspected to be a major source of abduction loss. This suspicion has led to significant research focused on the muscular process and its role in maintaining abduction.[79,83-85] This has included evaluation of the effect of age, placement site, implantation technique and various suture configurations.[79,84,85,90] Most of this research has been performed in single cycle to failure models and has uniformly been associated with loads at failure that are much higher than those demonstrated to occur *in vivo*. In one study, six different suture configurations in the muscular process of the arytenoid were examined using mechanical testing.[85] It was concluded that sutures that sufficiently engaged the spine of the muscular process alone or in conjunction with a second suture were the most biomechanically stable. These results parallel research in dogs that found the size of the hole for the suture or the suture size had no or little effect, but that sutures incorporating the arcuate crest were biomechanically superior, overall.[83] Recently, transection of the CAD muscle immediately behind its insertion onto the muscular process has been reported to allow access to debride the cricoarytenoid (CA) joint.[74] Transection of the CAD at its insertion also allows for superior identification of the muscular process and possibly for more accurate and consistent suture placement as a result.

The method of suture placement into the arytenoid cartilage has historically not been shown to be an important factor (e.g. tunnelling a hole with a hypodermic needle compared to a swaged on needle).[84] However, the use of a conventional cutting needle compared to a tapercut needle resulted in significantly lower peak loads at failure in a single cycle model and resulted in significantly more distraction in a cyclical model.[93] This was thought to result from the cutting needle creating a groove into which the suture propagated more readily than when placed with the tapercut needle. Recently, a screw placed into the

arytenoid has been utilised as a point of anchorage in the arytenoid.[71] This technique has not been utilised widely, however it is thought to increase stability of the arytenoid segment of the laryngoplasty construct. The use of such a suture anchor has been demonstrated to reduce the load required to achieve effective arytenoid abduction by approximately 50%.[91] Regardless of the method of attachment to the arytenoid, based on the cyclical testing that has been performed it appears that the arytenoid cartilage is unlikely to be a major source of loss of abduction if appropriate attachment is achieved.[92]

1.8.2 The cricoid cartilage

More recently, the cricoid cartilage has been identified as an important component that may be a source of variability and potential laryngoplasty failure. Computed tomographic imaging of the cricoid has found there to be notable conformational differences among cricoid specimens from a group of Thoroughbred larynges.[33] Logically, this variability at the caudal aspect of the cricoid cartilage may result in some variability in the ability of the cricoid to maintain a suture in the laryngoplasty construct. Suture migration in the cricoid was identified as the cause of 50% of short term excessive loss of abduction in when it occurred in a series of 200 horses.[68]

Various techniques associated with the cricoid cartilage have been proposed to reduce the loss of abduction. These range from sawing the suture to ‘seat it’ prior to tying, placing the sutures ‘into’ the dorsal notch when present, double loops around the caudal edge of the cricoid and novel laryngoplasty systems involving cables and washers or anchors.[75,80]

1.8.3 The cricoarytenoid articulation

The cricoarytenoid articulation (CA) is an important component of a laryngoplasty construct. The distances from the longer corniculate process to the articulation and

subsequently to the shorter muscular process are important components of a lever arm. As a result of this lever arm, small changes in the position of the muscular process will result in proportionally much larger changes in the positioning of the corniculate process. For example, a loss of 5mm between the arytenoid/suture/cricoid construct will lead to a 15-20mm loss at the corniculate process and resultantly significant abduction loss.[93]

Recently the effect of stabilising the CA by injecting polymethylmethacrylate into the joint space was evaluated using a static airflow model. [93] It was found that stabilising the joint with PMMA resisted collapse of the airway and reduced the force experienced by the suture during maximal abduction. More recently, a surgical technique to promote ankylosis of the CA has been published that resulted in decreased loss of abduction of the arytenoid in the post-operative period.[74] Utilisation of a technique to ankylose the CA joint reduced the forces on the laryngoplasty sutures and resulted in less loss of abduction post-operatively and should be considered as a means to help reduce loss of arytenoid abduction.[74,93,94]

1.8.4 The prosthetic suture/material

Various prosthetic materials have been reported to successfully perform a laryngoplasty. Materials reported are not limited to but range from catgut, wire, nylon, polyester, polytetrafluoroethylene, ultra-high molecular weight polyethylene, to braided cable.[66-68,73,75,77,78,88,95,96] Many of these materials are much stronger than the necessary requirements to obtain and maintain abduction. It is broadly accepted that a non-absorbable material that is of sufficient size (e.g. #5 [7.0metric]) is very unlikely to itself fail mechanically and cause of loss of abduction in an equine laryngoplasty construct. The strength of these materials is so far greater than the loading seen in a laryngoplasty construct *in vivo* that so long as a reasonable suture material is selected it will not be a

cause of laryngoplasty failure.[97] It has been found that the use of two sutures in the construct is superior to a single strand.[85] This is likely due to increased cartilage stability with two strands rather than any increased strength on the part of the suture. Additionally, slightly different angles of the sutures have been suggested to increase the CA joint stability *in vitro*.[98]

1.9 Conclusions

The currently available laryngoplasty techniques are fundamentally based on achieving a static partially abducted position of the arytenoid. This permanently abducted position is inevitably a compromise between the two major functions of the larynx: airflow and deglutition. As a procedure, it has numerous and varied complications generally associated with under abduction and reduced airflow or over abduction and contamination of the airway. These complications are mostly associated with the static nature of the laryngoplasty. The inability to determine where the "ideal" degree of abduction is at the time of surgery and the inability, regardless of technique, to reliably position the arytenoid without loss of abduction are limitations of the current laryngoplasty technique. With these points in mind the overarching goal of this research was to develop a laryngoplasty procedure that would be able to modify the degree of abduction of the arytenoid after surgery. The ability to modify the degree of arytenoid abduction post-operatively may potentially allow for improved success rates and lower complication rates as a result. As such, this project attempts to develop such a system for use in horses.

1.10 Project Aims and Hypotheses

The principle aim of this project is to *develop* and *evaluate* a dynamic laryngoplasty system (DLPS) for use in horses.

The major hypotheses to be tested are:

1. The developed dynamic laryngoplasty system (DLPS) is able to produce and maintain a variable degree of arytenoid abduction *in vitro*
2. *In vivo*, the DLPS will allow for controlled variation of the arytenoid position post-operatively

To test these hypotheses the following key objectives were set, each forming major themes of the project:

Objective I: Prototype conceptualisation, development and optimisation

a) Prototype conceptualisation and development
b) *In vitro* comparison of three techniques of prosthesis attachment to the muscular process of the equine arytenoid cartilage

This objective involved the development of a variety of working concept systems using 3D printed models. Each system was evaluated for functionality using cadaveric larynges with modifications and variations made to develop the final DLPS prototype that could be used to cause controlled alterations in arytenoid abduction. Once the DLPS had been developed to a working version the suture loop in which the device would be placed was examined in an effort to maximize the mechanical advantage of its positioning minimising the force required to achieve arytenoid abduction. This involved comparing 3 methods of attachment to the arytenoid cartilage and selecting the most mechanically advantageous for use in the final DLPS system.

Objective II: *In vitro* evaluation of the developed DLPS

a) Mechanical testing of a prototype DLPS to cause functional shortening of a suture loop
b) *In vitro* evaluation of the efficacy of a prototype DLPS to affect arytenoid abduction using equine cadaveric larynges
c) Evaluation of a prototype DLPS *in vitro* using a static airflow model

This objective involved three stages of *in vitro* testing for the DLPS. The first step was basic mechanical testing to establish performance under static load, cyclical and ramp testing situations. Second, the prototype was evaluated using cadaveric larynges to evaluate the ability to affect arytenoid abduction within the specific anatomy of cadaveric equine larynges. Finally, the prototype was evaluated using a static airflow model to ensure the device could maintain the previously demonstrated arytenoid abduction when under pressures similar to those seen in exercising horses.

Objective III: Proof of concept study

a) *In vivo* evaluation of a prototype DLPS in horses

This third and final objective was to confirm the surgical deliverability of the DLPS and evaluate the degree of surgical arytenoid abduction that could be achieved using the DLPS at 7-days post-operatively.

PART II: PROTOTYPE CONCEPTUALISATION, DEVELOPMENT AND OPTIMISATION

Chapter 2: Prototype development

2.1 Introduction

When considering the overarching goal of developing a device that could allow for alteration in the degree of arytenoid abduction at a point postoperatively, a number of factors were considered. To achieve this goal a device would need to be “activated” in some way that would cause an effective change in the degree of abduction of the arytenoid. When examining the four key components of a traditional laryngoplasty, namely the cricoid, the arytenoid, the cricoarytenoideus joint and a suture loop, the portion that could potentially be modified to achieve the above stated goal was considered to be the suture loop element. The suture loop is a flexible component of the laryngoplasty and if this could be functionally shortened at a point post-operatively then this would achieve the goal of selective alteration of arytenoid abduction. The development of a prototype to achieve this functional shortening is the subject of the remainder of this chapter.

2.2 Conceptualisation

To achieve the required functional shortening of the suture loop various options were considered with a focus on developing a simple and robust method. A balloon placed between the suture loops was considered as means to achieve this shortening. Such a positioned balloon would cause the suture loop to diverge and shorten as a result, which should cause alteration in the degree of arytenoid abduction (Figure 2.2.1).

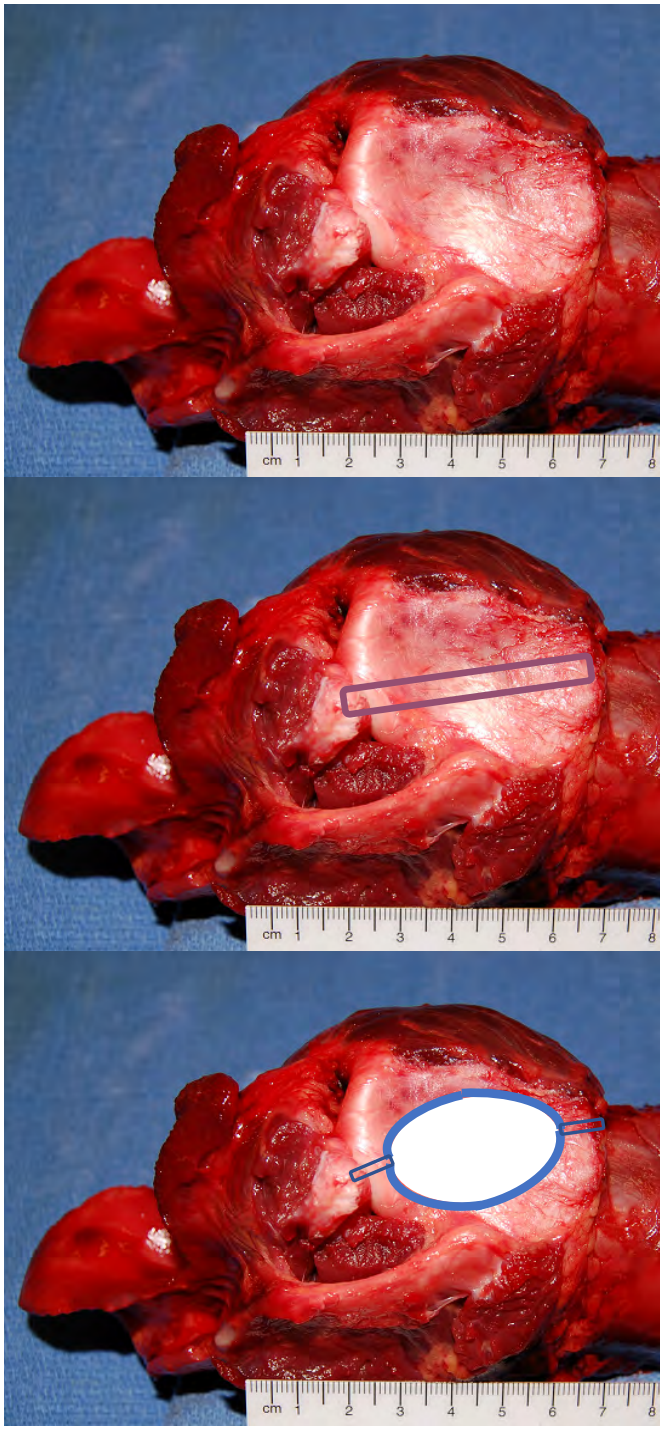

Figure 2.2.1 Left view of the larynx with the left CAD muscle removed (top). A stylised traditionally placed suture loop (middle) from the muscular process of the arytenoid (left) to the dorsocaudal aspect of the cricoid (right). The conceptualised balloon location between the suture loop that would result in functional shortening and increased arytenoid abduction as a result (bottom).

However, maintaining the suture loop between the inflating balloon to cause the shortening was considered to be a problem. To restrain the suture relative to the balloon two parallel layers of a stiff material that would resist the expansion of the balloon and further direct this expansion such that it would cause divergence of the previously parallel suture loop was considered. The original prototype used two discs of plastic either side of a balloon such that inflation caused the balloon to expand in a controlled fashion (Figure 2.2.2)

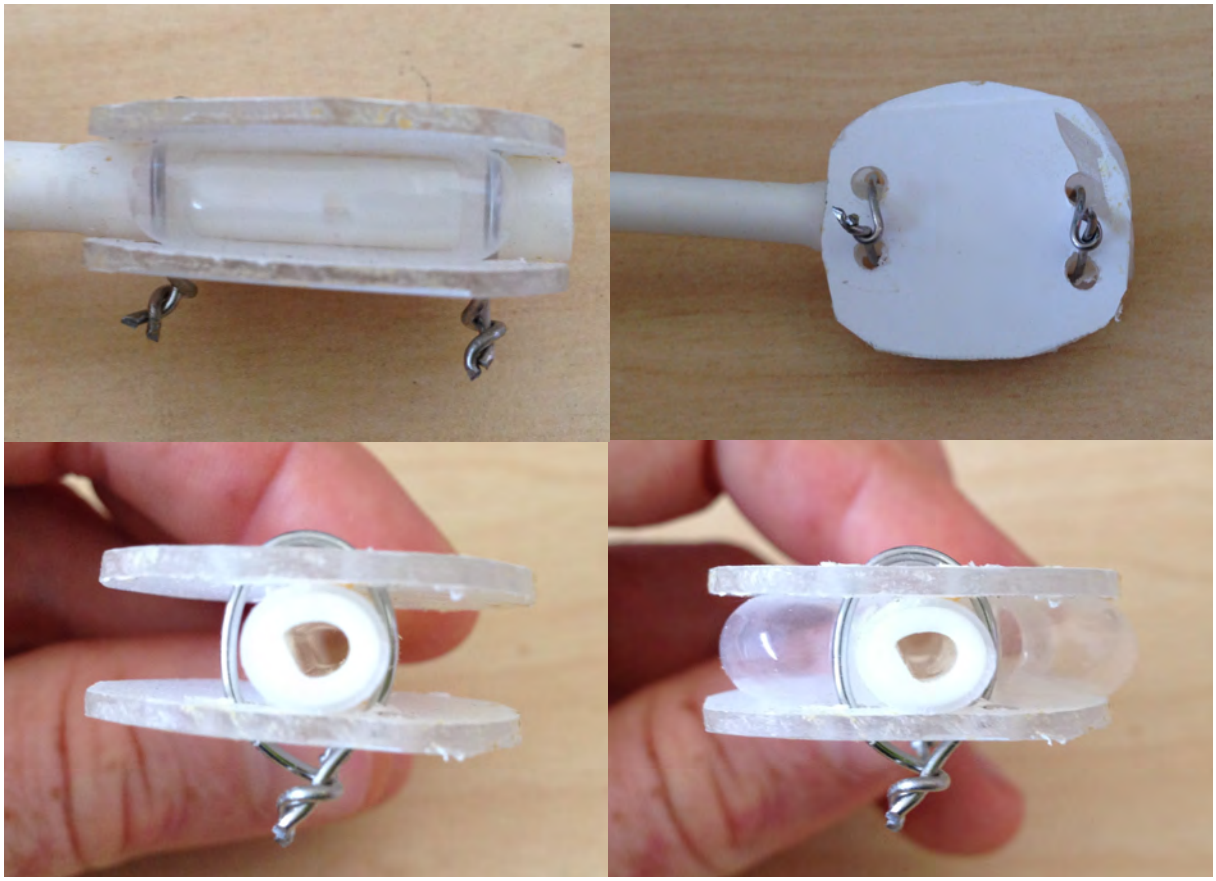

Figure 2.2.2 The original prototype used two parallel discs of plastic (side view – top left, top view – top right) that constrained the balloon within the device (end on view non-inflated state – bottom left and end on view inflated state – bottom right).

The developed device was then placed within the suture loop such that in a non-inflated state it caused minimal interference with the suture loop but when inflated it caused the

suture loop to diverge and as a result cause functional shortening. The suture loop, where in contact with the balloon, had a tube of stiff plastic placed over it to prevent the suture from sliding past the expanding balloon during inflation. The diameter of the stiff plastic tube was slightly less than the width between the plastic discs (Figure 2.2.3).

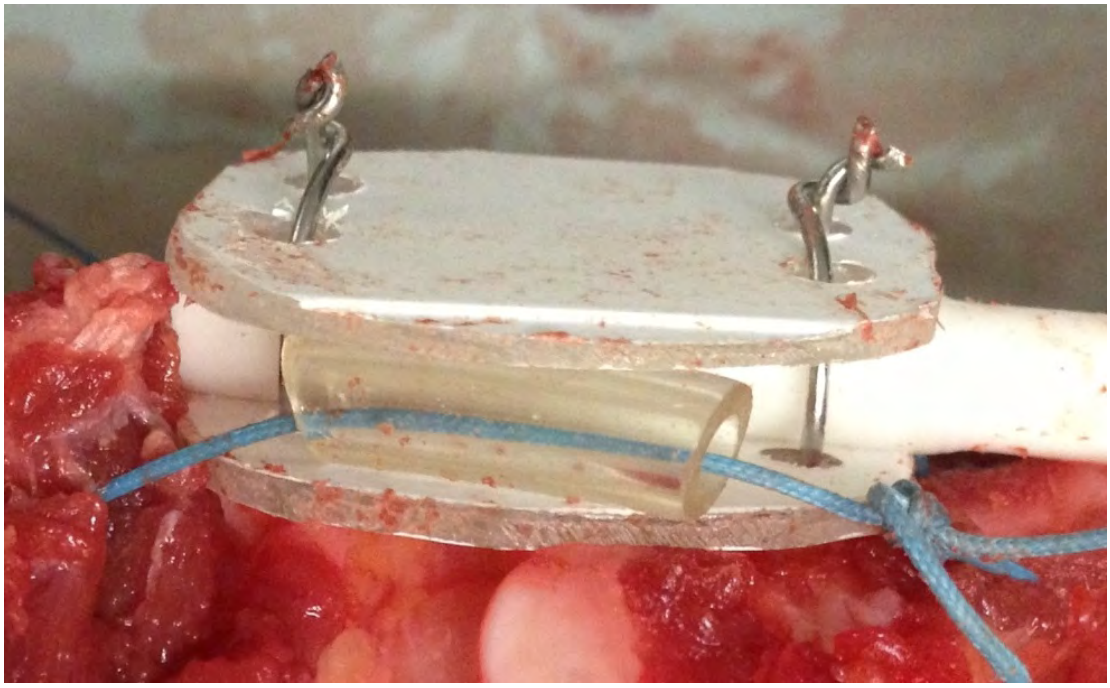

Figure 2.2.3 The original prototype demonstrating a suture loop with the stiff plastic tubing in place over the suture where it is in contact with the balloon.

This original prototype was effective at causing an increase in arytenoid abduction when inflated between a suture loop in a cadaveric larynx. However, this original device was large and cumbersome and additional prototype designs were developed.

2.3 Prototype versions

Online computer-aided design (CAD) software (www.tinkercad.com) was utilised to create precision drawings of potential models for evaluation. The models were then printed using a 3D printer in a variety of different materials, the idea being to develop the original

concept device outlined above into a refined prototype for further evaluation. In total eight different design options were developed and printed using 3D printer with three considered options demonstrated in figure 2.3.1 below.

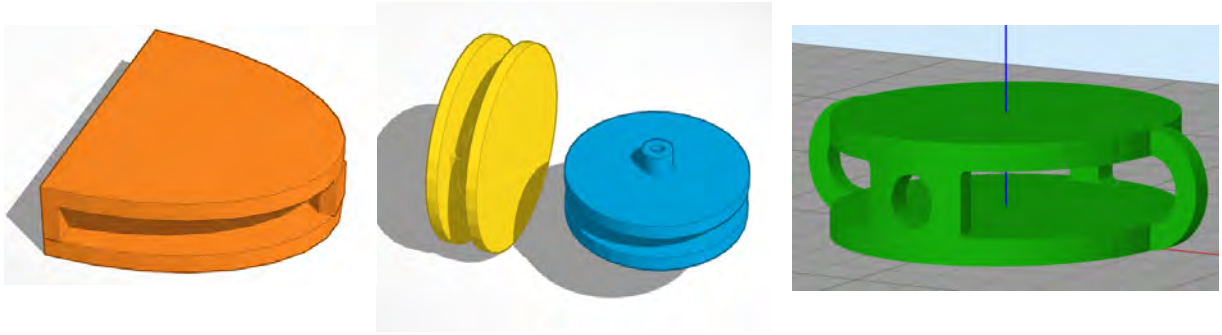

Figure 2.3.1 Three options developed using CAD software and printed using a 3D printer for evaluation.

Four types of materials that are available for use in 3D printing were evaluated. These included nylon, Avrylonitrile Butadiene Styrene (ABS), Polylactic acid (PLA) and Polymax™ PLA. These materials are thermoplastics that are used in 3D printing and have been used medical implants. The first three materials were found to be too soft and easily deformed with little force and as such were not able to constrain the balloon as required to achieve the required function. The Polymax™ PLA is significantly stiffer than the other materials but quite brittle such that when the balloon inflated the pillars connecting the two discs would break (Figure 2.3.2).

Figure 2.3.2 A polymax™ PLA 3D printed prototype showing the failure point during evaluation with a balloon inserted and inflated.

The 3D printed versions were extremely useful in determining the dimensions of the device that was most appropriate to fit the anatomic limitations of the equine larynx. A material that was considerably stronger and stiffer than the 3D printed options was required. Polyether ether ketone (PEEK) is a colourless organic thermoplastic polymer that is used in engineering applications and also for non-metallic surgical implants. This material is commonly engineered to specifications from a block of PEEK in small prototypes or by liquid mould injection in larger batches. The final version based on evaluation of the 3D models was selected and engineered from PEEK for further testing. The selected version using CAD modelling is demonstrated in figure 2.3.3 below.

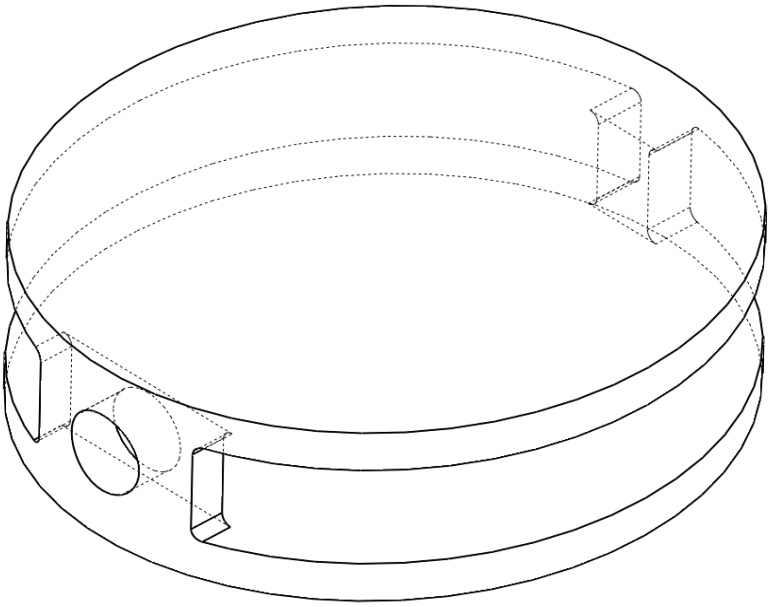

Figure 2.3.3 model selected for manufacturing from PEEK material for *in vitro* evaluation.

2.4 Prototype evaluations

The final selected version made from PEEK was tested in a small number of cadaveric larynges to determine the general function of the device prior to further more stringent evaluations. The PEEK, unlike the previously evaluated 3D printed materials, did not deform or break with balloon inflation and resulted in effective increases in arytenoid abduction when positioned between a suture loop (Figure 2.4.1).

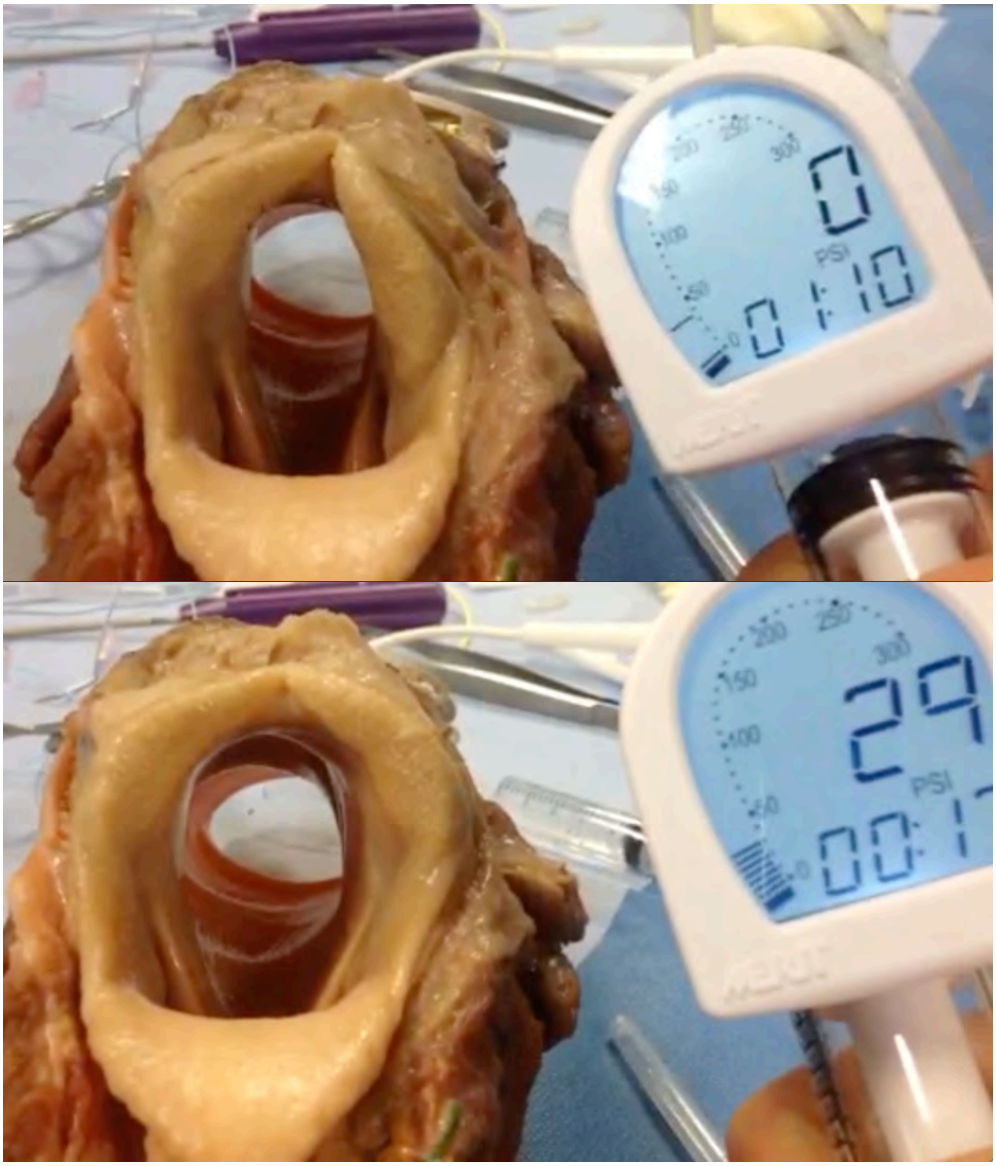

Figure 2.4.1 Preliminary evaluation of the PEEK prototypes ability to cause increased arytenoid abduction with balloon inflation (no inflation – top image and inflated to 29psi using a pressure syringe – bottom image).

2.5 Cadaveric testing of deliverability / functionality

Using an entire equine cadaver, the deliverability and functionality of the developed prototype was evaluated. Using a standard laryngoplasty surgical approach for access to the dorsolateral aspect of the larynx the prototype was inserted (Figure 2.5.1).

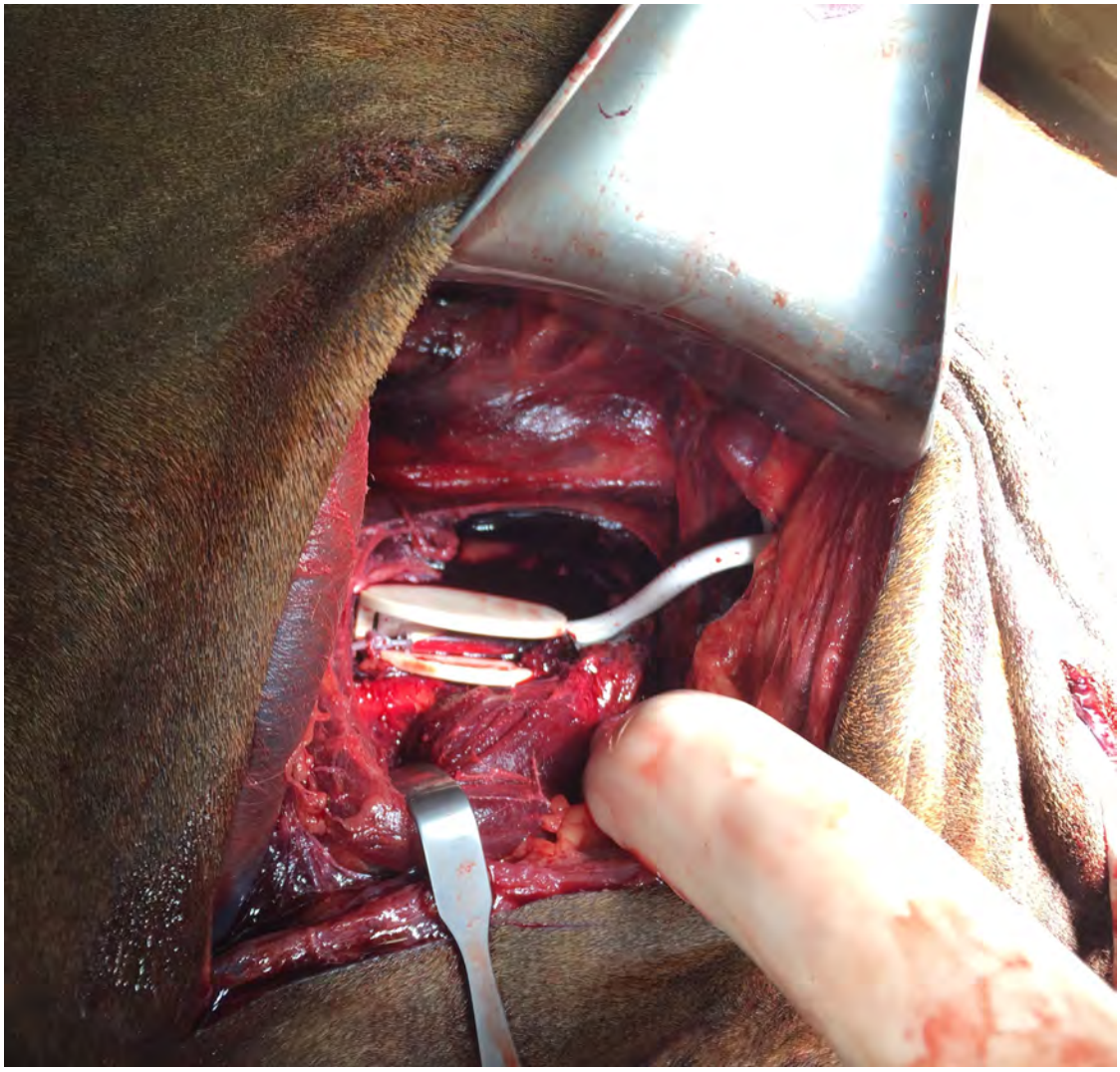

Figure 2.5.1 Surgical approach to the dorsolateral aspect of the larynx demonstrating effective deliverability of the prototype device in an equine cadaver.

Subsequently, an endoscope was placed via the right nostril to allow for visualisation of the rima glottis. Inflation of the balloon component of the prototype resulted in increased arytenoid abduction (Figure 2.5.2).

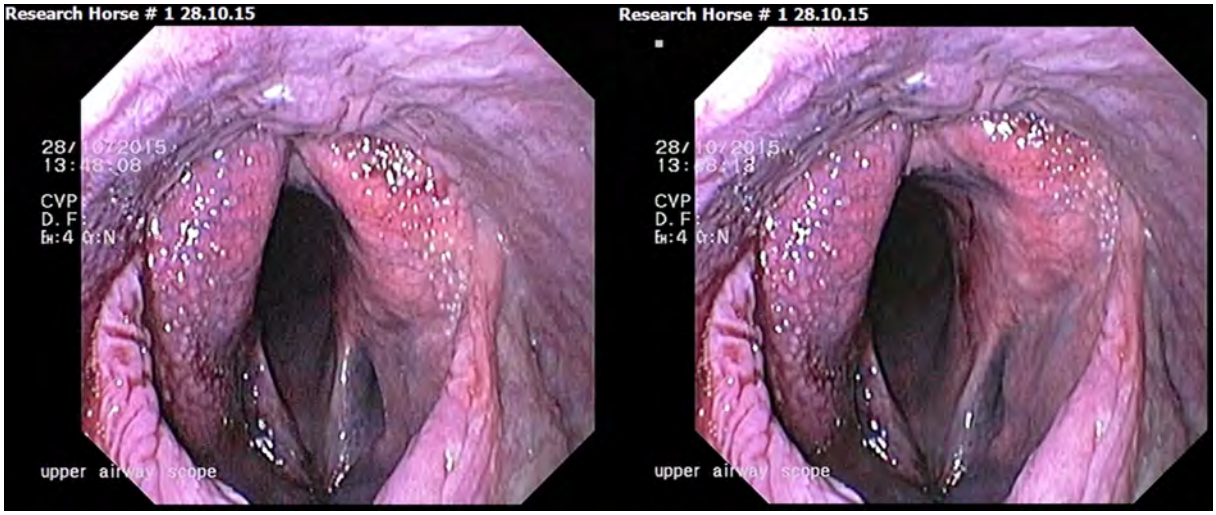

Figure 2.5.2 Videoendoscopic images of pre-inflation (left) and post-inflation (right) states using the PEEK prototype selected for further evaluation.

2.6 Discussion

When considering the goal of developing a device that could allow for alteration in the degree of arytenoid abduction at a point postoperatively a number of factors were considered. To achieve this goal a device would need to be “activated” in some way that would cause an effective change in the degree of abduction of the arytenoid. The suture loop is a flexible component of the laryngoplasty and if this could be functionally shortened at a point post-operatively then this would achieve the goal of selective alteration of arytenoid abduction. With these considerations in mind we developed and evaluated a variety of prototypes and different methods of attachment to the arytenoid before the final version of the DLPS was selected for subsequent testing.

Chapter 3: *In vitro* comparison of 3 techniques of prosthesis attachment to the muscular process of the equine arytenoid cartilage

Statement of authorship

Title of Paper	*In vitro* comparison of 3 techniques of prosthesis attachment to the muscular process of the equine arytenoid cartilage
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Principal Author

Name of Principal Author	Benjamin J. Ahern	
Contribution to the Paper	Designed experiments (80%) Performed experiments (100%) Wrote the paper (100%) Performed statistical analyses (50%)	
Overall percentage (%)	90%	
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.	
Signature		Date 20/6/17

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Andrew W. Van Eps		
Contribution to the Paper	Critically reviewed paper (50%)		
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Name of Co-Author	Raymond C. Boston		
Contribution to the Paper	Performed statistical analyses (50%)		
Signature		Date	20/6/17

Name of Co-Author	Samantha H. Franklin		
Contribution to the Paper	Designed experiments (20%) Critically reviewed paper (50%)		
Signature		Date	20/06/17

3.1 Abstract

Objectives- Biomechanical comparison of arytenoid abduction achieved using three different methods (FASTak II suture anchor and 2 suture patterns) of prosthesis attachment *in vitro* to determine which achieves the greatest abduction at various clinically relevant loads.

Study Design- Experimental.

Study population- Cadaveric larynges from 10 Standardbred racehorses.

Methods- Each larynx was sequentially instrumented with all three options of arytenoid suture attachment in random order and then underwent mechanical testing at varying loads. Each construct was abducted at 5 N increments from 0 to 25 N and the left to right quotient angle ratio (LRQ) measured from digital pictures acquired at each sequential increment.

Results- The FASTak II construct achieved significantly increased arytenoid abduction (higher LRQ) than both of the suture patterns tested. The largest difference occurred at 5N. There was no significant difference between the two suture constructs tested at any load point.

Conclusions- Use of the FASTak II suture anchor resulted in a construct that was biomechanically superior to the 2 suture patterns tested. This is likely due to the anchor producing a greater moment of force around the cricoarytenoid joint compared to the suture patterns.

Clinical Relevance- Use of the FASTak II anchor requires less suture load to achieve arytenoid abduction *in vitro* and should occur similarly *in vivo*. This will reduce the load on the laryngoplasty construct in general and may result in less abduction loss post-operatively.

3.2 Introduction

Recurrent laryngeal neuropathy (RLN) is a common problem in horses. The prevalence varies with breed with rates as high as 10% in Thoroughbreds and 42% in draft horses.[26,31] The general principle of the laryngoplasty technique has undergone remarkably little variation since it was first described by Marks et al. in 1970 and is still considered to be the treatment of choice for this condition in athletic horses.[65] Nevertheless, there is a high incidence of complications, including complete failure of abduction due to acute construct failure (3-11%) or gradual loss of abduction, which is reported to occur in nearly all cases in the longer post-operative period.[99-102]

Numerous models of single cycle and more recently cyclical testing have attempted to identify components of the laryngoplasty construct that can be improved in order to achieve a rigidly stable degree of rima glottis abduction.[84,90,92,103-105] Furthermore, multiple novel prostheses that focus on reducing suture/cartilage failure have been developed and evaluated since Marks first described the laryngoplasty,.[71,73,75,80,96,105] These all have similar biomechanical fundamentals, being the placement of a prosthesis of some type from the caudal cricoid to the muscular process tied to cause arytenoid abduction.[106] The orientation and positioning of the prosthetic material at the caudal aspect of the cricoid cartilage has been examined to evaluate which configuration or positioning achieves the best biomechanical construct and hence arytenoid abduction.[84,98,107,108] Many studies have focused on different methods of anchoring the respective prosthesis to the muscular process mostly in single cycle to failure types of testing.[79,84,85] One study compared two techniques for suture placement in the muscular process using single cycle testing and found no significant differences between direct suture placement using a trocar needle and through a predrilled hole.[11] Another, more recent study by Lechartier et al (2014), examined the mechanical properties of three different anchoring tests in the muscular

process of the arytenoid and found that a single screw technique (Corkscrew® device, Arthrex) was more stable than a double loop and at least as stable as a single loop of suture in cyclical testing.[25] Additionally, the double loop was found to be the strongest, followed by the single screw then the single loop in single cycle to failure. It was concluded that the anchor used was a reasonable option to hold loads in the arytenoid without failure to a similar level as the more commonly used suture materials.

The focus of the research we report here was to evaluate three different methods of muscular process attachment in order to determine the most mechanically advantageous method to achieve arytenoid abduction. We utilised a FASTak II® suture anchor with #2 Fiberwire® preloaded (Arthrex). This anchor is similar to the one used by Lechartier et al but has a slightly narrower diameter. Specifically, we wanted to determine which of the three constructs required the least amount of force to achieve clinically relevant arytenoid abduction.

The aim of this study was to objectively assess the effect of 3 different methods of arytenoid attachment, 2 suture patterns and one FASTak II anchor, on rima glottis area in equine cadaveric laryngeal specimens. We hypothesized that the FASTak II anchor would achieve greater arytenoid abduction than both of the suture patterns evaluated due to a superior mechanical position on the lever arm (muscular process) of the arytenoid. Furthermore we hypothesized that the more commonly recommended suture direction of caudomedial to craniolateral[107] would result in a greater arytenoid abduction than a medial to lateral directed suture selected to represent what may be the “least ideal” position option.

3.3 Materials and methods

3.3.1 Specimen preparation

Larynges from 10 Standardbred horses aged 3-11 years euthanized for reasons other than respiratory disease were collected and frozen at -20^{0}C. Larynges were thawed in batches at room temperature for 24 hours. The thyroid, cricoid, epiglottic, arytenoid cartilages and the right cricoarytenoideus muscle were all that were retained for testing. All other soft tissue including the left cricoarytenoideus dorsalis muscle was removed.

3.3.2 Specimen positioning

Each larynx was positioned in exactly the same way, in a customized cradle positioned 30cm from a digital camera (HC-V250 high definition digital camera, Panasonic, Osaka, Japan) that was controlled remotely using generic wireless technology. [84,98,107] A 10cm ruler was affixed to the cradle in frame as a reference.

3.3.3 Suture placement

For each larynx, the right side was abducted routinely using suture material and fixed in an abducted position for the remainder of the testing. Subsequently, the left side of each larynx was instrumented with each of the three constructs in a random order. Both suture constructs were placed using #5 Ethibond Excel (7 metric; V-37, Ethicon Inc, NJ)[92] in either a caudomedial to craniolateral or horizontal medial to lateral direction. The FASTak®II (Arthrex) with preloaded #2 Fiberwire suture (AR-1324HF, Arthrex) was placed in the arytenoid 10mm cranial to the insertion of the CAD muscle, as described previously.[25] The free ends of the 6 cm long loops were then attached caudally, immediately adjacent to the cricoid cartilage 1.5cm lateral to the midline, to a hand held tensiometer (Transducer techniques, Temecula, CA) similar to previous reports.[98,107,109]

3.3.4 Mechanical testing

Each construct was sequentially loaded from 0 to 25N at 5N increments with digital images acquired at each point. The tested construct was then removed and replaced by the next construct and repeated until all were tested.

3.3.5 Left to Right Quotient angle ratio (LRQ)

Arytenoid LRQ was measured and calculated, as described previously, from each photograph by one observer (B.A) using a soft-ware package (Image J, Bethesda, USA).[51,110] In brief, a line was drawn from the ventral to the dorsal aspect of the rima glottis and then extended by one third to give a point that was used to measure the left (L^O) and right (R^O) arytenoid angles. The LRQ was calculated by L^O divided by R^O.

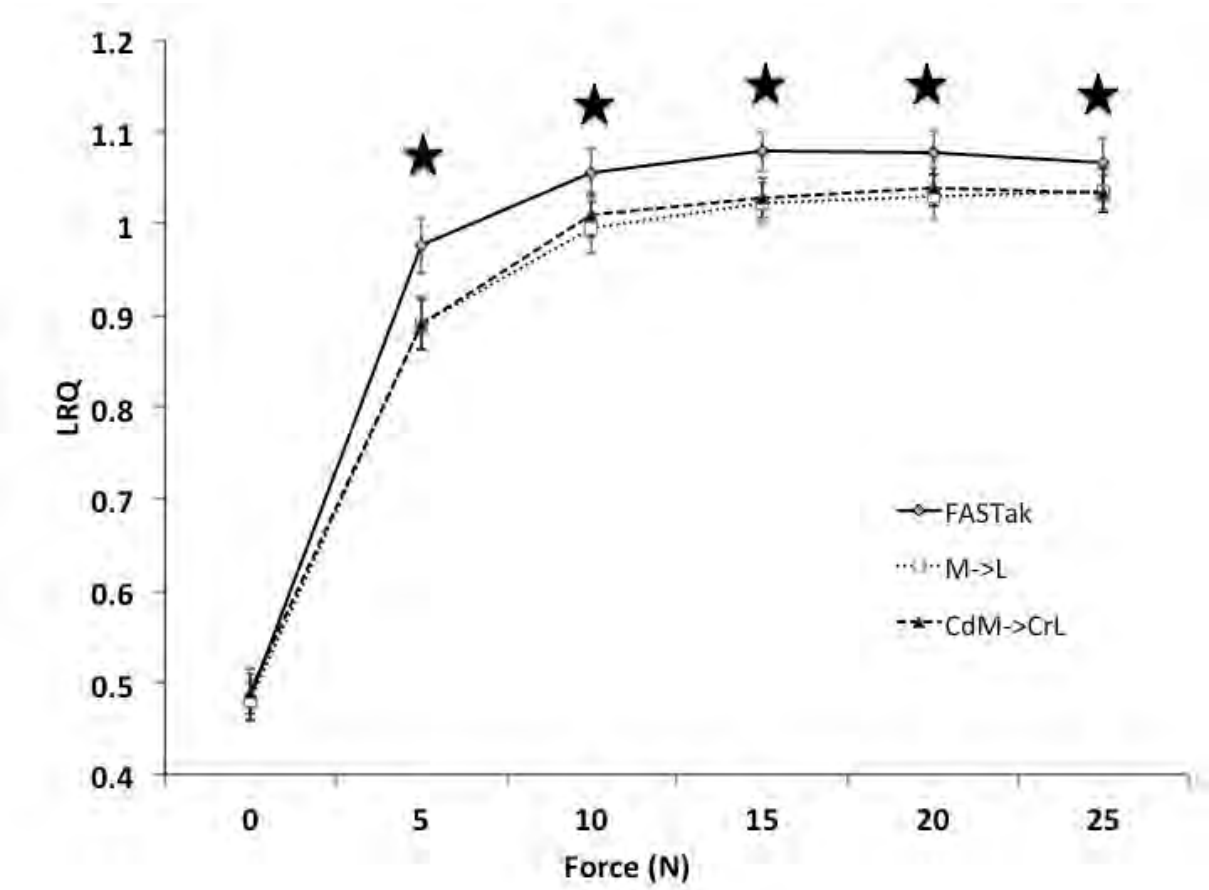
3.3.6 Statistical analysis

Data were checked for normality and homogeneity of variance using D'Agostino's SK test, '90, and Levene's, '60, Robust test for the equality of variances between groups. Data was modelled using mixed effects regression models with the outcome 'LRQ' and predictor variables 'load' and 'construct'. Wald statistics were used to indicate significant terms in the mixed effects model and a chi-square test was used to confirm the significance of the random effect. A p-value of 0.05 was used to assign statistical significance throughout.

3.4 Results

At zero load, there was no significant difference in LRQ between the three constructs (p=0.7) (Figure 1.1). The FASTak II construct produced significantly more abduction (higher LRQ) compared to both the suture constructs at all loads above zero with the

752 difference being largest at 5N ($p<0.02$). There were no significant differences between the
753 two suture constructs at any load throughout the study ($p>0.05$).
754



755

756 **Figure 1.1: Load versus resultant LRQ for each construct.** Stars denote significant
757 differences between FASTak II and suture constructs ($p<0.05$). (FASTak was the FASTak
758 construct, M->L was the medial to lateral suture placement and CdM->CrL was the
759 caudomedial to craniolateral suture placement)
760
761

762 ## 3.5 Discussion

763 The objective of this study was to determine which of the three prosthesis attachment
764 techniques evaluated produced the superior mechanical construct. Specifically, we

attempted to determine which construct produced the greatest arytenoid abduction at the lowest load. We proved one hypothesis that the FASTak II anchor would produce the highest LRQ and disproved our second hypothesis that the more commonly recommended caudomedial to craniolateral suture placement would be superior to a medial to lateral angled suture in the arytenoid.

The FASTak II anchor provides a point of attachment for the suture material that is abaxially located in the muscular process of the arytenoid as compared to the location of the suture patterns. Such positioning results in less force being required to abduct the arytenoid because of the effect of the lever arm created by the cricoid, arytenoid and cricoarytenoideus articulation.[30] This mechanically superior positioning coupled with the previously reported comparable overall holding strength of a similar type of anchor compared to suture constructs and ease of placement of the anchor (requires less dissection in clinical cases) makes its use to attach suture to the muscular process very attractive. *In vivo*, the use of the anchor should result in less force needing to be applied to the suture material to achieve the desired degree of arytenoid abduction and result in a reduced load on the construct in general. We hypothesise that this should lead to a reduced loss of abduction due to cartilage or suture failure.

The loads used in this study were similar to those previously reported when evaluating the cricoid cartilage component of the construct.[88,107] Whilst, the lower loads (5N) we reported to achieve abduction in cadaveric larynges may not transfer directly to the *in vivo* situation, the differences seen between the anchor and the suture techniques persisted to relatively high *in vitro* loads of 25N. This is not dissimilar to the load of 27.6N that has been reported to be required to achieve clinically relevant arytenoid abduction, *in vivo*.[88] It is reasonable to conclude, based on the findings of this study, that using the FASTak II anchor *in vivo* may result in a significant reduction in the load required to achieve

clinically relevant abduction. This may then result in less clinical abduction failure if there is less load on the prosthesis.

Gradual loss of arytenoid abduction in the post-operative period occurs in most laryngoplasty procedures.[99] Given this common complication, techniques have been developed such as mechanical arthrodesis of the cricoarytenoid joint which has been shown to result in less abduction loss post-operatively[74] Other techniques to stabilize the cricoarytenoid joint have also been reported using laser facilitated surgery or injection of polymethylmethacrylate. [93,94] These techniques focus on destroying the cricoarytenoid joint which is still an option when using an anchor such as the FASTak II and indeed combining the two may result in an even more stable laryngoplasty construct, although this needs to be further evaluated.

We tested the constructs similar to previously reported models of *in vitro* evaluation of arytenoid abduction rather than mount the constructs on a servo hydraulic testing system. This allowed for the angle of suture distraction to be controlled to very accurately represent the *in vivo* positioning, which would not have been as possible on a servo hydraulic testing system. Also, we chose to test each of the 3 constructs on each of the larynges to minimize variability in the testing system to allow for a clear understanding of changing the single variable of how the suture was attached to the arytenoid and what effect this had on arytenoid abduction. Finally, we measured the degree of abduction using LRQ rather than cross sectional area (CSA) as this has previously been shown to be a more accurate measurement tool *in vitro* compared to CSA.[110]

There was no difference between the 2 suture patterns tested. Retrospectively this is not surprising as the bites across the arytenoid, whilst in a different plane, still go across the body of the arytenoid and the summative effect of the suture loop through the arytenoid results, mechanically speaking, in the distraction force coming from the middle of the body

of the arytenoid. As a result, there was no difference between the two patterns in the ability to achieve arytenoid abduction. Clinically this means that the more important point during suture placement is achieving a secure bite with the suture and the direction that the suture is placed across the arytenoid is not important with regards to the load required for abduction.

Comparison between 3 different techniques of prosthesis attachment to the muscular process of the equine arytenoid cartilage found there to be significant differences in the load required to achieve arytenoid abduction.[25] In our study, we found that using an abaxially located anchor such as the FASTak II (Arthrex) resulted in a construct that achieved abduction whilst requiring the least load. Use of this, or similar, suture anchors should clinically result in it being easier to obtain the desired degree of intra-operative arytenoid abduction and result in less initial and ongoing load on the construct. This reduction in required load should result in a more stable laryngoplasty construct *in vivo* with less acute short term and gradual longer term loss of arytenoid abduction.

PART III: *IN VITRO* PROTOTYPE EVALUATION STUDIES

Chapter 4: Mechanical testing of a prototype dynamic laryngoplasty system (DLPS)

Statement of authorship

Title of Paper	Mechanical testing of a prototype dynamic laryngoplasty system (DLPS)
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Name of Principal Author	Benjamin J. Ahern		
Contribution to the Paper	Designed experiments (80%) Performed experiments (100%) Wrote the paper (100%) Performed statistical analyses (100%)		
Overall percentage (%)	90%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	20/6/17

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Andrew W. Van Eps		
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Name of Co-Author	Samantha H. Franklin		
Contribution to the Paper	Designed experiments (20%) Critically reviewed paper (50%)		
Signature		Date	20/06/17

4.1 Abstract

Objectives- *In vitro* biomechanical evaluation of a prototype dynamic laryngoplasty system (DLPS).

Study Design- Experimental.

Study units- Ten prototype DLPS constructs.

Methods- Ten DLPS constructs were evaluated using an Instron mechanical testing system. This included sequential testing in 3 stages to assess 1) the amount of distraction achieved by DLPS activation at a static 10N load, 2) the response to cyclical testing from 10 to 15N and 3) single cycle ramp to 100N.

Results- The DLPS prototype was able to cause 7.11±0.14mm shortening of a suture loop while under a static 10N load. During cyclical testing, no acute failures occurred and overall 0.16±0.03mm of construct lengthening was recorded. Nine of the 10 constructs completed a single cycle ramp loading to 100N without failure, the remaining one device failed at 38.6N.

Conclusions- The prototype was found to be able to cause an approximately 12% functional shortening of the suture loop and be able to withstand short term cyclical loading. Nine of the 10 DLPS prototypes withstood ramp loading considerably above that reported for *in vivo* loading on laryngoplasty sutures. Overall, the DLPS performed sufficiently to justify further evaluation both *in vitro* and *in vivo*.

Clinical Relevance- The potential to modify the degree of arytenoid abduction post-laryngoplasty may be a useful tool for equine surgeons considering loss of abduction post-operatively is currently a common complication.

4.2 Introduction

Recurrent laryngeal neuropathy (RLN) is a common problem in horses. The prevalence varies with breed, with rates as high as 10% in Thoroughbreds and 42% in draft horses.[26,31] The laryngoplasty (LP) technique described by Marks *et al.* in 1970 is currently the treatment of choice for RLN in racehorses.[65] Complications following LP in horses are many and varied and have been reported extensively.[99,102] With the current LP technique, it is a challenge during surgery to achieve the "ideal" degree of abduction to maximise respiratory function whilst avoiding complications associated with this static position of the arytenoid abduction. The basic premise of laryngoplasty is the use of a prosthetic material to pull the arytenoid into abduction and maintain it statically in that position. This underlying principle has not changed but there have been multiple variations developed and evaluated.[71,73,75,80,96,105] All of these LP system variations have been aimed at attempting to maximise the stability of the static LP construct (i.e. arytenoid abduction).

The focus of the research we report here was to evaluate a prototype dynamic laryngoplasty system (DLPS) *in vitro*. This DLPS was developed with the goal of being able to selectively alter the degree of arytenoid abduction in the horse after surgery. This device could have a number of advantages compared with the traditional LP. It could be used to optimise arytenoid cartilage positioning in the immediate post-operative period. Alternatively, it could be used to allow for correction of the gradual loss of abduction that occurs post-operatively.[111] Finally, it might allow the arytenoid to be maintained in a relatively adducted position at rest and be altered, to a maximally abducted position, when peak airflow is required during exercise. Overall, an option to alter the degree of abduction post-operatively may lead to reduced complications and potentially allow for improved respiratory function during maximal exercise.

The developed DLPS prototype allows for shortening of a traditional laryngoplasty suture loop by means of a balloon constrained within a custom designed polyether ether ketone (PEEK) device (Figure 1).

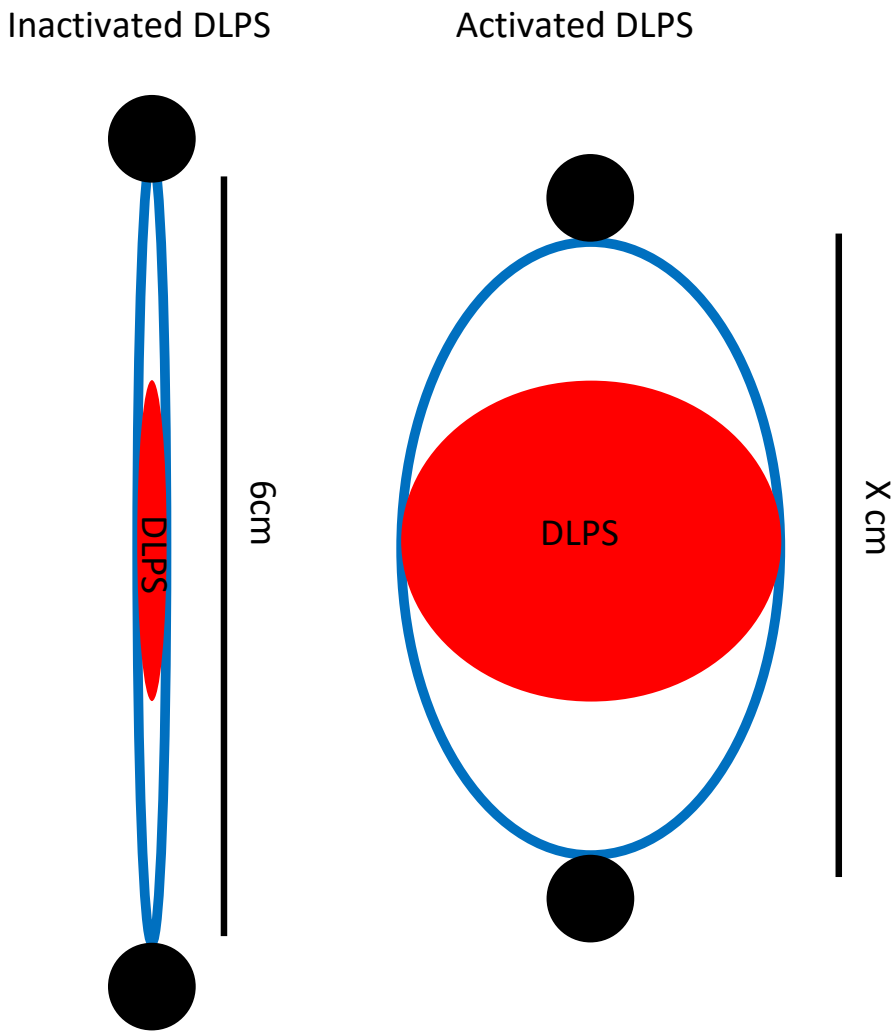

Figure 1. Diagram form of how the prototype functions by inflation of the balloon (red) between the 6cm long suture loop (blue) causing shortening as a result (functional shortening=6cm-Xcm). Black circles indicate points of attachment to mechanical testing machine.

The device is designed to have a minimal profile and dimensions to suit the anatomic site of placement: specifically, to fit within the space bounded by the caudal aspect of the cricoid and the muscular process in a fully abducted position and from the sagittal ridge of the cricoid to the wing of the thyroid cartilage. The DLPS can be inflated or deflated using saline injected via an attached catheter that could eventually be attached to a subcutaneous injection port allowing for minimally invasive post-operative alteration of arytenoid abduction.

Prior to further testing of the DLPS prototype we wanted to determine its performance using a mechanical testing unit. Our aims were to 1) determine the degree of construct shortening that was possible using the DLPS, 2) evaluate the DLPS during cyclical loading and 3) evaluate the DLPS during a single cycle to failure.

We hypothesised that the DLPS would 1) be able to effectively shorten the LP suture construct (greater than 10% shortening of the suture loop), 2) maintain construct shortening during cyclical testing (less than 1% increase in suture loop whilst the device is activated) and 3) not fail during single cycle ramp testing.

4.3 Materials and Methods

Ten DLPS constructs were evaluated. All testing was performed using a servohydraulic testing machine (Instron, Norwood, MA) linked to a data acquisition program (LabChart™). For each test a new single strand of #2 Ethibond Excel ([5 metric] Ethicon Inc, NJ) was used to form a 6cm loop (total suture length 12cm) which was passed through 2 metal eyelets rigidly affixed to the loading arms of the mechanical testing machine and tied using standard technique to mimic a laryngoplasty suture loop. The selected DLPS prototype was then instrumented in the 6cm loop and connected to an adjustable pressure

syringe (Blue Diamond™ Merit Medical) (Figure 2). Each DLPS construct was sequentially tested in 3 stages; 1) static load-distraction, 2) cyclical testing and 3) single cycle ramp to 100N.

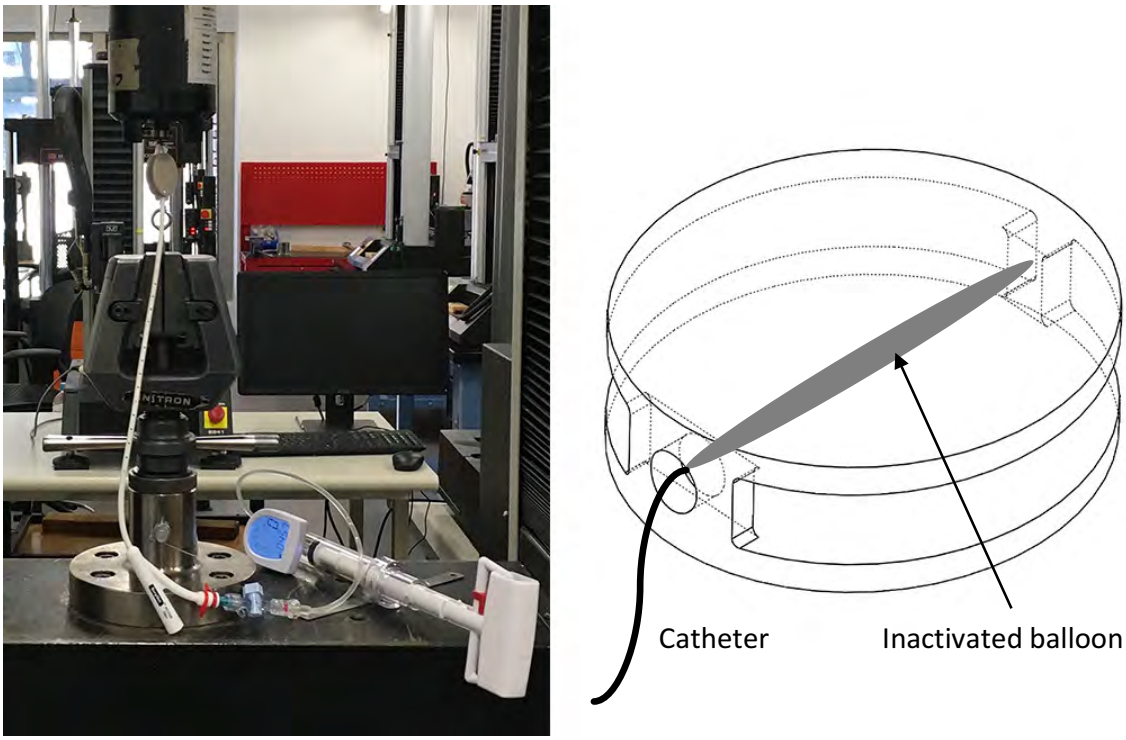

Figure 2. Picture on left demonstrates construct positioning for testing and the PEEK component of the DLPS in schematic form on the right.

4.3.1 Static load – distraction testing

Each DLPS construct was loaded to 10N and maintained at this load. The loss of distraction (construct shortening) required to maintain the target 10N load caused during sequential DLPS activation at 10psi increments to a maximum of 60psi as measured using the adjustable pressure syringe were recorded.

4.3.2 Cyclical testing

With the DLPS activated at 50psi the load on the construct was cycled from 10 to 15 N at a rate of 1 cycle per 1 second (1Hz) for 30 minutes (total 1800 cycles). The distraction that occurred over the duration of this testing was recorded.

4.3.3 Single cycle testing

With the DLPS activated at 50psi the load was ramped to failure or 100N load (whichever occurred first) at a rate of 0.83mm/s (50mm/min). From the generated load-distraction curves, the load at failure (N) and stiffness (N/mm) were calculated for each construct.

All data is reported as mean ± SEM throughout unless otherwise stated.

4.4 Results

4.4.1 Static load – distraction testing

All constructs completed testing without failure at the target load of 10N. The DLPS caused a maximal shortening of the construct length of 7.11±0.14mm (Figure 3). This equates to an effective 11.85% shortening of the 60mm long suture loop by maximal activation of the DLPS. Further pressure increases were not possible due to the suture material disengaging with the DLPS device or prolapsing off the DLPS balloon around the suture.

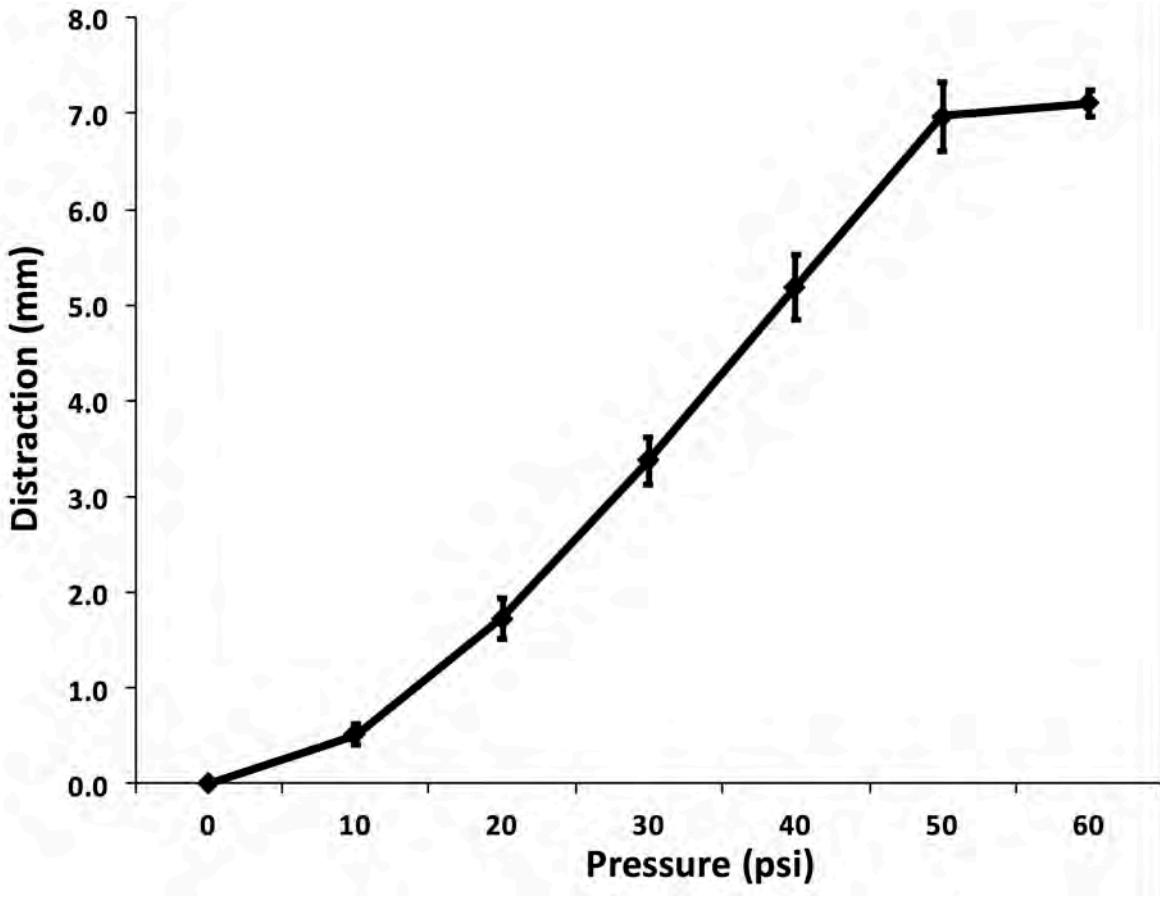

Figure 3. Mean (+/- SEM) Distraction achieved during static load testing of 10 DLPS constructs at 10psi increments.

4.4.2 Cyclical testing

All constructs completed testing without failure. Over the 1800 cycles (30 minutes at 1Hz) there was an average increase of 0.16±0.03mm in construct length. The minimum and maximum load during testing was tightly controlled and was on average 9.82±0.10N and 15.37±0.28N respectively.

4.4.3 Single cycle testing

Nine of the 10 DLPS constructs were loaded to 100N without failure and the testing was stopped due to load cell restrictions at that point. The stiffness for these 9 constructs was

11.35±0.63N/mm. The remaining single DLPS construct failed due to balloon rupture at 38.6N and most likely occurred due to the balloon pinching against the DLPS implant.

4.5 Discussion

We evaluated a novel DLPS *in vitro* using a series of mechanical tests to determine the performance of this device in a simulated equine laryngoplasty suture loop. The DLPS was able to cause shortening of the suture loop, withstand cyclical loading for 180 cycles and 9 of the 10 DLPS withstood 100N of load without failure. Based on this *in vitro* evaluation further evaluation of the DLPS is warranted.

Our first goal was to determine what degree of shortening was possible using a suture loop similar to a laryngoplasty construct *in vivo* using the DLPS. A 6cm loop of suture was selected based on previous measurements of 10 adult equine cadaveric larynges which had an average cricoid length at the dorsal midline of 5.3±0.7cm resulting in a laryngoplasty suture loop length of approximately 6cm (Ahern, unpublished data). The selection of a static force of 10N to test the degree of distraction that the DLPS could generate was based on previous *in vivo* and *in vitro* work.[88,89,112,113] The force on the laryngoplasty suture *in vivo* when placed with the animal under general anaesthesia has been reported to be 27.6±7.5N.[88] However, recently during standing laryngoplasty the force required to obtain abduction was subjectively lower in comparison.[72,88] *In vitro,* much lower force (14.7N in one study) has been reported to be required to achieve arytenoid abduction.[89] These reported forces were measured using a traditional laryngoplasty suture placement through the muscular process. The use of a suture anchor to position the point of distraction in a mechanically superior position (more abaxially) will likely result in less force being

required to achieve effective abduction and, as a result, 10N was selected for this testing.[113] Based on these parameters the DLPS was able to cause 7.11mm of shortening, or almost 12% reduction of the overall length of the construct.

The second goal was to evaluate the DLPS prototype during cyclical loading. The cyclic load was from 10 to 15 N at 1Hz. This rate of 1Hz was selected to ensure the Instron machine maintained a high level of accuracy at these relatively low loads. Using this cyclical testing for a period of 1800 cycles was completed successfully with a very small amount of total distraction (0.16mm or 0.26% of overall length). This number of cycles was similar to previously reported for *in vitro* laryngoplasty testing with the rate of loading slightly lower.[75] Laryngoplasty sutures have been shown to undergo loading a mean of 1152 times in a 24 hour period.[88] As a result, the 1800 cycles used for this *in vitro* testing equates to slightly more than 1.5 days of cyclical testing seen *in vivo*. Using this *in vitro* model of testing the prototype was able to withstand cyclical loading sufficiently in the short term to justify further research.

The third and final goal was to evaluate the prototype during a single cycle test to a 100N load which is a considerably higher load that is likely to occur *in vivo* at any point.[88] One prototype failed at 38.6N and this appeared to be due to pinching of the balloon component of the DLPS against the PEEK. Smoothing of the PEEK portion of the device for mild manufacturing blemishes will likely resolve this problem. Another factor to consider is that using the DLPS prototype, the *in vivo* maximal load may not be as high as the previously reported 46.6N (recorded during induced swallowing) due to differences in the way the laryngoplasty was performed between these studies. Specifically, the use of an anchor for the arytenoid attachment has been shown to reduce the force required to achieve abduction

by 50% compared to a traditional suture placement.[91] Following on from this reduced load to achieve abduction it is possible that the peak load may be lower than the reported 46.6N. If the peak load is indeed lower than the 46.6N as a result of using the anchor then the DLPS failure that occurred at 38.6N is likely to be above the expected *in vivo* loading. As such, all 10 DLPS devices would have failed at loads above the expected *in vivo* conditions. However, the maximal *in vivo* load using an anchor as a component of a laryngoplasty is unknown and would be an interesting direction of future research.

Laryngoplasty techniques based on the procedure originally developed by Marks *et al* in 1970 have undergone extensive *in vitro* and *in vivo* evaluation. The underlying principle of placing a prosthetic material(s) from the muscular process of the arytenoid to the caudal aspect of the cricoid has remained uniform throughout. Variations have been many and varied from originally Lycra to various types of suture material and even zip-ties.[65,73,80,105] The laryngoplasty has been associated with success rates ranging from 45-70% in flat racehorses.[57,67,77,114] The most common and significant complications associated with the procedure are loss of post-operative abduction and airway contamination (coughing / dysphagia).[99,102] The development of a laryngoplasty system such as the DLPS evaluated in this research should as a minimum allow for post-operative surgical abduction modification / maintenance or ideally the ability to change the degree of arytenoid abduction longer term based on the specific exercise requirement of a horse at any particular time.

This research evaluated the mechanical function of a prototype DLPS *in vitro* to determine the device's ability to cause functional shortening of a simulated laryngoplasty construct and withstand physiologically relevant loading. The results of this research indicate that

the further development and evaluation of the DLPS as a means to achieve dynamic arytenoid positioning is warranted.

Chapter 5: *In vitro* evaluation of the effect of a prototype dynamic laryngoplasty system (DLPS) on arytenoid abduction

Statement of authorship

Title of Paper	*In vitro* evaluation of the effect of a prototype dynamic laryngoplasty system (DLPS) on arytenoid abduction
Publication Status	☐ Published X ☐ Submitted for Publication ☐ Accepted for Publication ☐ Unpublished and Unsubmitted work written in manuscript style
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Principal Author

Name of Principal Author	Benjamin J. Ahern		
Contribution to the Paper	Designed experiments (90%) Performed experiments (50%) Wrote the paper (95%) Performed statistical analyses (100%)		
Overall percentage (%)	80%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	20/6/17

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Yee Wei Lim		
Contribution to the Paper	Performed experiments (50%) Wrote the paper (5%)		
Signature		Date	20/6/17

Name of Co-Author	Andrew W. Van Eps		
Contribution to the Paper	Critically reviewed paper (50%)		
Signature		Date	20/6/17

Name of Co-Author	Samantha H. Franklin		
Contribution to the Paper	Designed experiments (10%) Critically reviewed paper (50%)		
Signature		Date	20/6/17

5.1 Abstract

Objectives- To evaluate the ability of a prototype dynamic laryngoplasty system (DLPS) to affect arytenoid abduction *in vitro*.

Study Design- Experimental.

Study population- Cadaveric larynges from 10 adult Standardbred and Thoroughbred racehorses.

Methods- Dissected larynges were mounted and the right arytenoid maximally abducted for subsequent testing. A left sided laryngoplasty was performed using a single strand of #2Fiberwire and a FASTak®II suture anchor. Phase 1 of testing involved progressively shortening (tightening) the suture, without the DLPS device in place, in 1 mm increments and acquiring a digital image of the *rima glottidis* at each increment. Phase 1 was used to assess the amount of shortening required to achieve full arytenoid abduction and as a frame of reference for the results of subsequent testing. Phase 2 involved completing the laryngoplasty (tying the suture) at a target left to right quotient (LRQ) angle of 0.7 with the DLPS in place. Subsequently, digital images at 3 stages of DLPS activation (0, 25 and 50 or maximal psi when 50 was not achieved) were obtained. All digital images were stored and analysed to generate LRQ angles as a measure of arytenoid abduction.

Results- One larynx during dissection was found to have laryngeal dysplasia and was excluded. The remaining 9 larynges were anatomically normal and all aspects of testing were completed. In Phase 1, a total shortening of 25.89±1.27mm was possible and this increased the LRQ from 0.59±0.02 to a maximum of 1.07±0.12. In Phase 2, activation of the DLPS caused the LRQ to increase from 0.70±0.05 to a maximum of 0.97±0.09. This change in LRQ equated to 18.7mm of effective shortening based on Phase 1 results. The maximum psi before the balloon element of the DLPS prolapsed was 37.33±5.96.

Conclusions- The DLPS was able to increase the degree of arytenoid abduction *in vitro*. This increase in LRQ was equivalent to 18.7mm of effective shortening of the laryngoplasty suture loop based on Phase 1 of the study.

Clinical relevance- The DLPS may allow for adjustment of the degree of arytenoid abduction post-operatively. The results presented here support further evaluation of the device *in vitro* and ultimately *in vivo*.

5.2 Introduction

Recurrent laryngeal neuropathy (RLN) is a common problem in horses.[26,31] The traditional laryngoplasty (LP) technique was first described by Marks *et al.* in 1970 and is still considered to be the treatment of choice for this condition in athletic horses.[65] There is a relatively high incidence of complications associated with this procedure, which have been associated either with under or over abduction of the surgically abducted arytenoid.[99-102] Furthermore, some degree of failure as measured by loss of arytenoid abduction has been reported to occur in nearly all cases.[111] The most commonly reported complications related to over abduction include coughing and/or aspiration.[68,99,102]

The surgical procedure of LP as described by Marks *et al.* (1970) has undergone remarkably little variation when compared to the modern reported variations. Overall the common principle of all traditional laryngoplasty techniques is to replace the action of the cricoarytenoideus (CAD) muscle to achieve arytenoid abduction using single or multiple prostheses. Over the years there has been considerable research into and variation of various components of the laryngoplasty procedure with the research focusing on achieving static abduction of the arytenoid with no or minimal loss of abduction in the post-operative period.[71,73-75,80,96,105,112]

The overarching objective of our research was to develop a means for performing a laryngoplasty with the ability to alter the degree of arytenoid abduction selectively after surgery. With this aim in mind we developed a dynamic laryngoplasty system (DLPS) that we have evaluated *in vitro* via mechanical testing. This testing found the DLPS when activated was able to produce 7.11mm (12%) of suture loop shortening at a static 10N load. Furthermore, the DLPS was able to withstand 100N ramp loading without failure in 9/10 tests. Finally, cyclical testing resulted in 0.16mm (0.26%) of distraction loss during 1800 cycles.[115] Based on these encouraging mechanical testing results, the next step and

the focus of this research was to evaluate the ability of the DLPS prototype to achieve changes in arytenoid abduction *in vitro* using cadaveric larynges.

There were two aims of this study. The first aim (Phase 1) was to evaluate the relationship between shortening of the laryngoplasty suture (without the DLPS in place) and LRQ change, with the goal of assessing the amount of shortening required to achieve full arytenoid abduction and as a frame of reference for comparison with the results from the second phase of the study. The second aim (Phase 2) was to evaluate the relationship between DLPS activation and LRQ change.

We hypothesised that activation of the DLPS would cause a change in LRQ of greater effect than the equivalent of LRQ change caused by 7.11mm of construct shortening.

5.3 Materials and Methods

5.3.1 Specimen preparation and positioning

Larynges from 10 Standardbred and Thoroughbred horses aged 3-11 years euthanized for reasons other than respiratory disease were collected and frozen at -20^{0}C. Larynges were then thawed at room temperature for 24 hours immediately prior to testing. The thyroid, cricoid, epiglottic and arytenoid cartilages and their intrinsic muscles were retained for testing, with the other soft tissue removed. The cricoid length at the dorsal midline was measured using callipers and recorded for each larynx. Each larynx was positioned in a standardised manner: mounted in a customized cradle as previously reported[98] and positioned 30 cm from a digital camera (HC-V250 high definition digital camera, Panasonic, Osaka, Japan) that was controlled remotely using generic wireless technology. A 10-cm ruler was affixed in the same plane as the laryngeal opening as a reference in the digital image.

5.3.2 Laryngoplasty suture placement

For each larynx, the right arytenoid was maximally abducted and fixed in position for all testing as reported.[91,116] The left side had a #2 Fiberwire suture (removed from the preloaded position in a FASTak®II (AR-1324HF, Arthrex)) placed through the cricoid cartilage 1.5cm lateral to the midline and 1.5cm from the caudal edge. For the arytenoid attachment the FASTak®II suture anchor was placed in the arytenoid 10mm cranial to the insertion of the CAD muscle as previously described. [91,113] The suture was then threaded through the eyelet of the anchor from medial to lateral such that the two threads ends were in a relatively lateral position creating a single suture loop.

5.3.3 Phase 1 testing – suture shortening

For Phase 1 testing, the ends of the suture at the mid-point (from cranial to caudal) of the cricoid cartilage were attached to the arms of a Vernier calliper to allow for progressive controlled construct shortening at 1 mm increments by opening the calliper arms (Figure 1). Digital images were obtained at each 1mm of construct shortening to assess progressive arytenoid abduction until no further abduction could be achieved.

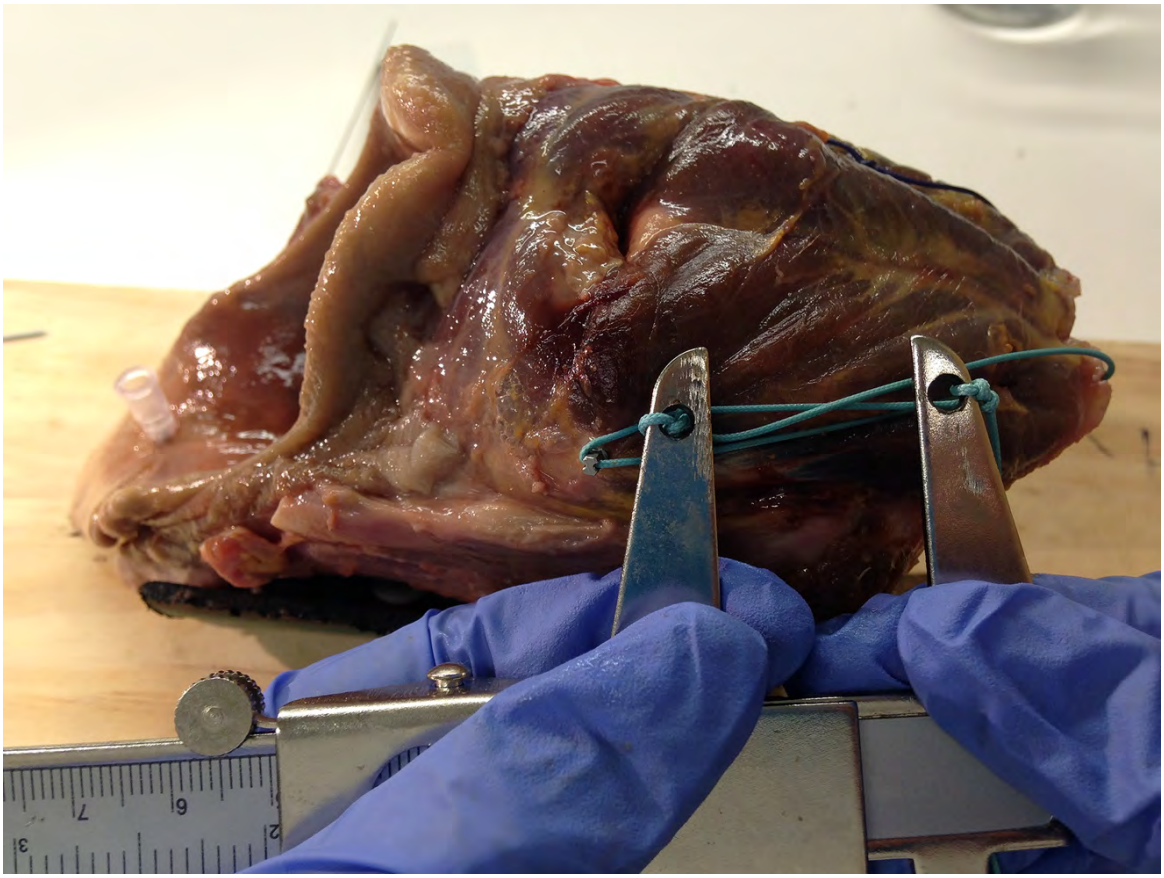

Figure 1. Position of callipers to allow for progressive shortening of the laryngoplasty construct.

5.3.4 Phase 2 testing – DLPS activation

Immediately following Phase 1 testing of each larynx, the suture was tied routinely with a surgeon's knot and 4 single throws to the left arytenoid at an approximate LRQ of 0.7. Subsequently, the DLPS was inserted in position between the two suture threads. The associated 30cm long catheter with the attached injection port was located so that handling of the injection port did not alter the position of the larynx. Three states were tested; "inactivated" (no saline injection - 0 psi) and 2 levels of activation (25psi and 50 psi). The 25 psi and 50 psi pressures of activation were achieved by injecting saline into the injection port and the pressures were selected based on the previous mechanical testing results.[115] As previously reported, the injection pressure was measured using a pressure

syringe (Blue Diamond™ Merit Medical).[115] If a pressure of 50psi was not achieved the maximal pressure was recorded and the reason for failure to achieve 50psi recorded. Digital images were obtained at each of the 3 levels (Figure 2).

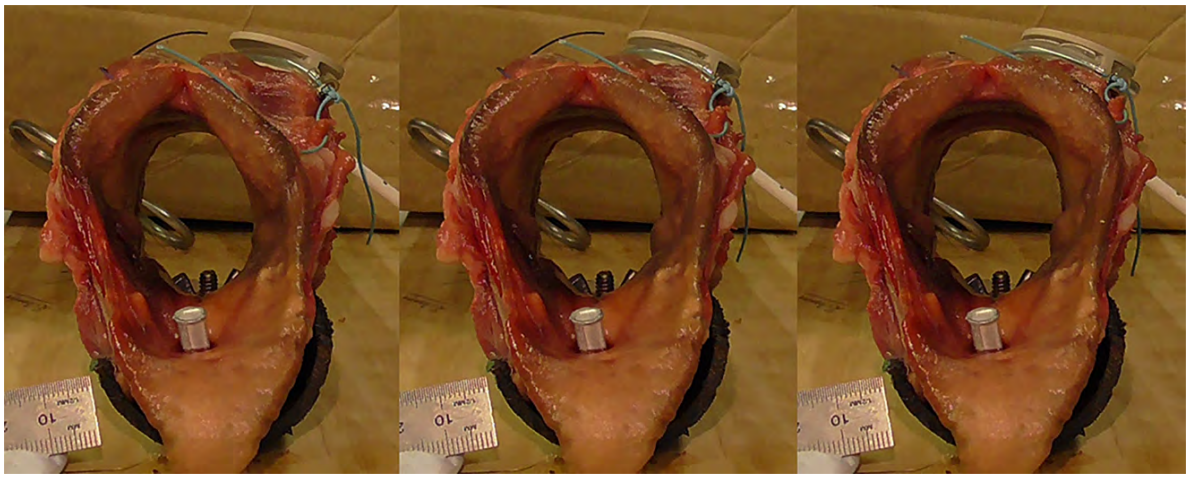

Figure 2. Larynx mounted for testing showing 0, 25 and 50 psi activation of the DLPS from left to right respectively.

5.3.5 Left to Right Quotient angle ratio (LRQ)

Arytenoid LRQ was measured and calculated as previously described from each photograph by one observer (B.A.) using a soft-ware package (Image J, Bethesda, USA).[51,110] In short, a line was drawn from the ventral to the dorsal aspect of the rima glottis and then extended by one third to give a point that was used to measure the left (L^O) and right (R^O) arytenoid angles. The LRQ was then calculated by dividing L^O from R^O.

5.3.6 Statistical analysis

Multivariable logistic regressions using ordinary least squares were used to a) fit the LRQ to amount of laryngeal shortening (mm) and b) fit the LRQ to DLPS pressure (psi) for each larynx used. Predicted estimates of the observed values were produced and used to calculate the line of best fit with 95% confidence intervals. All analyses utilised a 5% two-

tail probability point as the basis for rejection of null hypotheses of no difference from a zero value (slope). Stata version 14.1 was used for all analysis and data is reported as mean ± SD. Graphs are presented with 95% confidence intervals.

5.4 Results

During dissection one larynx was found to have abnormal anatomy consistent with laryngeal dysplasia and was excluded from testing. All remaining 9 larynges were anatomically normal and completed testing without problems associated with mounting, image acquisition or LRQ calculation. The 9 larynges had a dorsal cricoid length of 52.28±2.36mm.

For phase 1 of testing a total shortening of 25.89±1.27mm was achieved which resulted in the LRQ increasing from a baseline of 0.59±0.02 to a maximum of 1.07±0.12. This was a mean increase in LRQ of 0.47±0.12. The linear regression for all data points produced a R^2 value of 0.91 (Figure 3). A maximum of 25.89±3.82mm of suture shortening was possible before further shortening did not cause additional increases in LRQ.

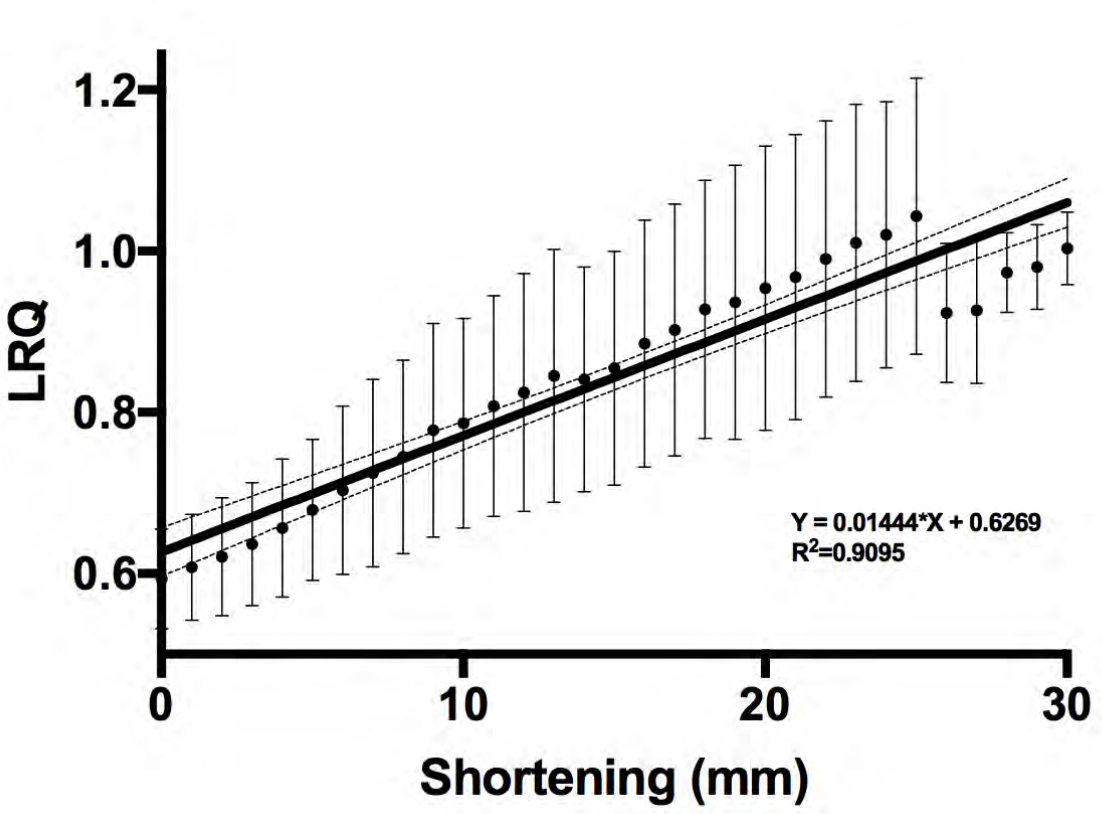

Figure 3. Linear regression of progressive suture shortening and resultant LRQ. Data reported as mean ± SD with 95%CI.

For phase 2 of testing, the DLPS was instrumented to the left side of the larynx between the two suture loops without complication in all cases. Activation of the DLPS resulted in an increase in LRQ from 0.70±0.05 (0 psi) to a maximum of 0.97±0.09 ($p<0.001$) (Figure 4). This change in LRQ equated to 18.7mm of effective shortening based on linear regression of the phase 1 results. Eight of the 9 DLPS failed to reach the target of 50 psi because in all cases the balloon component became overinflated and prolapsed from the device after protruding from the rigid portion of the DLPS. Additional injections of saline did not result in further increases in LRQ or pressure once the prolapse occurred. The maximum psi for all prototypes tested was 37.33±5.96 psi.

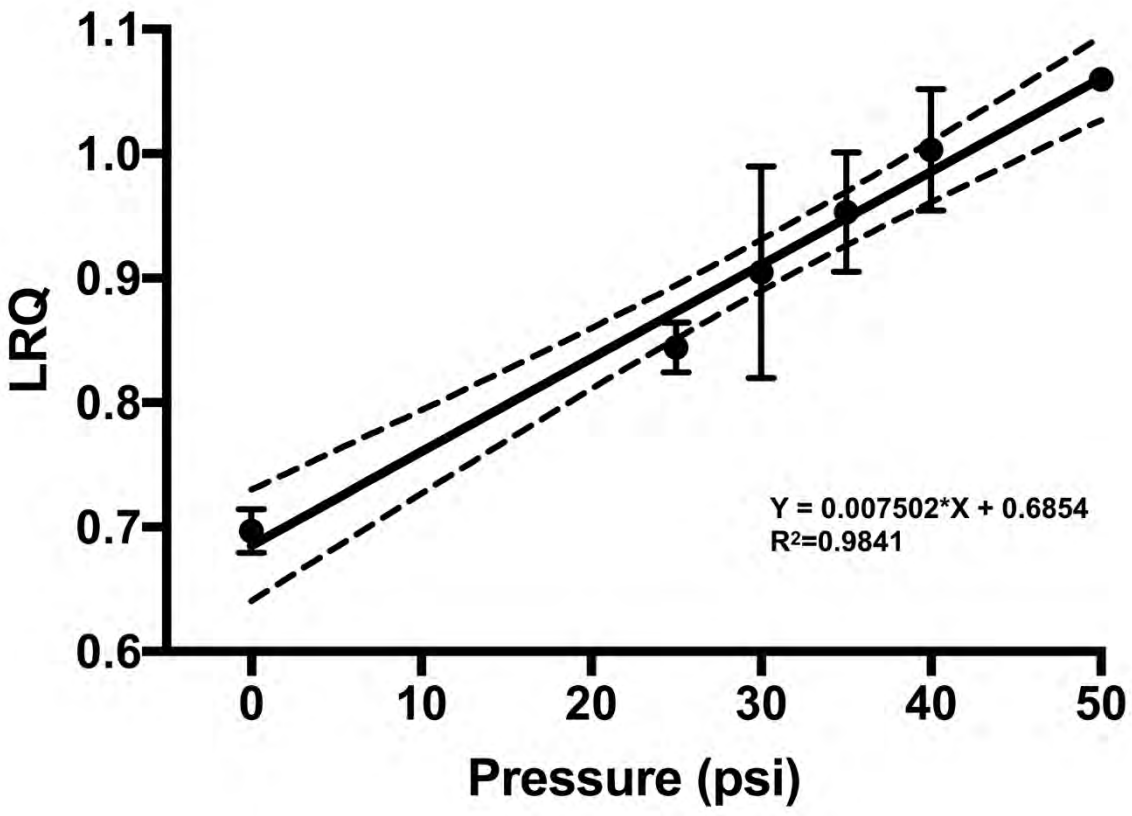

Figure 4. Linear regression of activation pressure and resultant LRQ. Data reported as mean ± SD with 95%CI.

5.5 Discussion

We evaluated *in vitro* a novel DLPS that allowed for selective alteration of the degree of arytenoid abduction using inflation via an injection port. This device has been developed as a potential method that could be used to selectively alter the degree of arytenoid abduction after laryngoplasty surgery. This option may be useful to optimise post-operative arytenoid abduction and potentially reduce complications associated with suboptimal (over or under) abduction.

This *in vitro* evaluation was essential to confirm the deliverability of the device and relative performance using cadaveric larynges. Previous mechanical testing evaluation of

the DLPS found that the device could cause functional shortening of 7.11m of a suture loop under a static load of 10N. Furthermore, the device was able to withstand ramp to 100N failure in 9/10 tests and had minimal displacement with cyclical testing.[115] This research aimed to build on these earlier mechanical testing results by evaluating the DLPS using cadaveric larynges. This phase of evaluation of the DLPS was important to ensure the device could cause alteration in arytenoid position in normal cadaveric larynges.

The 9 larynges used in this study were obtained from adult Thoroughbred and Standardbred horses with no visual evidence of laryngeal abnormalities. We elected to use LRQ as a means of evaluating change in arytenoid abduction similar to previous studies.[51,74,94,110] We selected LRQ because as long as the camera position and laryngeal position are fixed and uniform, as was the case in this testing, then minor variations in the original position of the larynx or camera do not affect the results. A similar means of *in vitro* testing using LRQ has been previously reported.[91,110] Cross sectional area of the rima glottis would have been an alternative method of measuring arytenoid abduction comparatively but based on previous research comparing the two methods there is no significant difference and due to the simplicity and robustness of LRQ measurements this was selected.[51]

The first phase of this study was performed to assess the amount of shortening required to achieve full arytenoid abduction and serve as a frame of reference for the results of phase 2 testing. During this phase 1, shortening of the suture loop using callipers without the DLPS in place, caused relatively consistent and linear (R^2=0.91) increases in LRQ up to between 25-30 mm of shortening. At that maximal degree of shortening further efforts to shorten the loop did not cause additional increases in LRQ and represented the maximal range of suture shortening possible which was limited by the relative anatomic dimensions of the larynx.

In the second phase of the study we evaluated the DLPS at three levels, based on the mechanical testing results to reflect what we considered to be baseline or inactivated (0psi), moderate activation (25 psi) and maximal activation (50 psi). We selected an inactivated target LRQ of 0.7 in an effort to represent a low to moderate level of arytenoid abduction that might represent the lower end of a spectrum of arytenoid abduction surgeons might aim for clinically when performing a laryngoplasty.[4,56,68] As a result, a LRQ of 0.7 was selected to represent a level at which the DLPS might be used clinically to increase arytenoid abduction post-operatively. This study evaluated the DLPS ability to cause this increase in LRQ from 0.7 with 2 levels of activation.

Activation by injection in the port was readily achieved using saline and caused significant increases in LRQ with increasing pressure. Using the linear regression model from Phase 1, the increase in LRQ by maximal activation corresponded with 18.7mm of suture shortening. This was more than the 7.11mm of suture shortening found in the previous mechanical testing study when tested at a static load of 10N. The result of this study supported our hypothesis that activation of the DLPS would cause a change in LRQ of greater effect than the equivalent of LRQ change caused by 7.11mm of construct shortening. The degree of LRQ change and corresponding suture shortening was considerably higher than during the previous mechanical testing. This is due to the cadaveric nature of this research where there are relatively little forces restricting arytenoid abduction. In an *in vivo* situation the arytenoid would be subject to adduction forces seen with coughing, swallowing or negative airway pressures which may mean the DLPS used *in vivo* may be less able to achieve the increases in arytenoid abduction seen in this *in vitro* study, although this is unknown at this time.[88] However, due to the use of an anchor in the arytenoid which has been shown to reduce the load on the laryngoplasty suture by approximately 50% the exact effect of these forces is as yet undetermined.[91] Before *in vivo*

testing, the next step in evaluating the DLPS should involve further *in vitro* testing using an airflow model, similar to previously reported studies, to simulate the negative airway pressures seen *in vivo*. [74,86,94]

The balloon component of the DLPS was found to prolapse and not cause further increases in LRQ at pressures between 30-40 psi. This was due to the DLPS having produced maximal shortening of the suture and represents the maximal amount of construct shortening achievable *in vitro*. As a result, it may be useful *in vivo* to monitor the injection pressure, or, the endoscopic appearance and when there is a plateau of pressure or in arytenoid abduction, then this will likely represent the point of maximum performance of the DLPS. With regards to monitoring the pressure the effect of soft tissue surrounding the DLPS *in vivo* is as yet unknown and may potentially limit prolapse of the balloon element, which may alter the pressure required to achieve full activation. As a result, the combination of endoscopic and pressure monitoring in conjunction may be the most ideal.

In conclusion, we found the prototype DLPS was able to effectively achieve significant increases in arytenoid abduction *in vitro*. We supported our hypothesis by finding the DLPS was able to cause arytenoid abduction equivalent to 18.7mm of shortening which was more than the 7.11mm found in previous mechanical testing research. These results support the further evaluation of this device with an overarching objective of trying to develop a clinically useful means of selectively altering the degree of arytenoid abduction post-operatively.

Chapter 6: Evaluation of a prototype dynamic laryngoplasty system (DLPS) *in vitro* using an equine vacuum airflow system

Statement of authorship

Title of Paper	Evaluation of a prototype dynamic laryngoplasty system (DLPS) *in vitro* using an equine vacuum airflow system
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Overall percentage (%)	80%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
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Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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6.1 Abstract

Objectives- Evaluation of a prototype dynamic laryngoplasty system (DLPS) *in vitro* using an static airflow model.

Study Design- Experimental.

Study units- Cadaveric larynges from 10 adult Standardbred and Thoroughbred horses.

Methods- Dissected larynges were mounted within the testing system with the right arytenoid maximally abducted for all testing. A left-sided laryngoplasty using a single strand of #2Fiberwire and a FASTak®II suture anchor was performed. Each larynx was tested using a static airflow model in 2 phases. Immediately prior to testing of each larynx, the system was adjusted to produce a standard 55 L/s flow rate and a pre-laryngeal pressure of 12 mmHg whilst both arytenoids were maximally abducted. For phase 1 of testing the suture loop was progressively shortened (tightened), in 3 mm increments from 0 mm to 30 mm and the larynx tested at each increment. In phase 2 the variable balloon portion of the DLPS was inserted and three stages of DLPS activation (inactivated [0], 25 and maximal psi) were tested. Each test was a 5 second period of static airflow as outlined above. Translaryngeal impedance (TLI), left to right quotient angles (LRQ) and cross sectional areas (CSA) were calculated for each test. Data is reported as mean +/- SEM.

Results- Activation of the DLPS caused increases in both CSA and LRQ ($p<0.05$). Translaryngeal impedance was significantly reduced by suture tightening up to 6mm, but not by additional shortening ($p=0.001$). In the second phase of the study, activation of the DLPS prototype caused a significant reduction in TLI from no activation (0 psi) (0.43±0.08mmHg/L/sec) to 25 psi (0.16±0.04mmHg/L/sec, $p<0.001$) and a smaller but non-significant additional reduction (0.13±0.03mmHg/L/sec) at maximal activation.

Conclusions- The prototype DLPS was able to cause significant reductions in TLI by activation from 0 to 25 psi and a further smaller non-significant reduction at maximal psi.

Clinical Relevance- The DLPS prototype in this *in vitro* airflow system was shown to be able to achieve and maintain arytenoid abduction that was beneficial to airflow. Based on these results further evaluation *in vivo* is supported.

6.2 Introduction

Recurrent laryngeal neuropathy (RLN) is a common problem in horses.[26,31] The currently recommended treatment of choice for this condition in athletic horses is a prosthetic laryngoplasty (LP) based on the technique originally described by Marks *et al* in 1970.[26,31,65] This LP technique fundamentally aims to produce static abduction of the arytenoid thereby preventing arytenoid cartilage collapse during exercise. Recent modifications of the LP have focused on different prosthesis materials, different methods to anchor to the cartilage, and evaluated different methods to fuse the cricoarytenoideus articulation.[71,74,93,94,113] Overall, the reported success rate for LP surgery for racehorses has been reported to be in the range of 38-78%.[106]

Our group has aimed to develop a LP technique that allows for alteration of the degree of arytenoid abduction post-operatively to maximise efficacy and minimise complications. This would ideally allow for a more dynamic, or adjustable, degree of arytenoid abduction that could be customised to a particular horses' airway requirements. This effort to develop an adjustable LP is based on the fact that when a LP is performed the majority of horses will develop a loss of arytenoid abduction by 1-2 grades within the first 6 weeks.[111] This loss of abduction can be quite variable and presents a challenge to surgeons who are aiming for the target degree of arytenoid abduction that equates to 88% of the cross sectional area of the *rima glottidis* based on computational modelling of airflow.[21] Based on previous *in vitro* testing by our group this suggested 88% CSA equates to an approximate LRQ of 0.7-0.75 (data not shown). To achieve these targets clinically it can be tempting to over-abduct the arytenoid at surgery and then rely on post-surgical abduction loss to occur and "settle" to the desired arytenoid position. However, complications such as coughing and aspiration pneumonia have been attributed to over abduction of the arytenoid.[73,78,88,99,117] Additionally, some of these complications such as

coughing have been found to put greater pressure on the prosthesis which can lead to either cartilage or LP failure.[99] Due to these considerations, the ability to alter the degree of abduction post-operatively to customise the degree of arytenoid abduction may provide a useful option for equine surgeons performing LPs.

Previously, our group has developed a prototype dynamic laryngoplasty system (DLPS) and evaluated it to ensure the device was capable of withstanding reasonable loading conditions whilst mounted on a mechanical testing machine. That research found the device was able to produce 7.11mm of construct shortening at a constant load of 10N, which was selected to approximate loading expected *in vivo* based on previous research. Subsequently, the DLPS was evaluated *in vitro* using cadaveric larynges to determine the ability of the device to alter the degree of arytenoid abduction within the specific anatomy of the equine larynx. That research found the prototype was able to achieve 18.7mm of effective shortening and significantly increase the degree of arytenoid abduction as a result.

The aim of this study was to evaluate the ability of the DLPS prototype to affect and maintain a reduction in translaryngeal impedance (TLI) using a static airflow model similar to previously reported systems, as a final step prior to *in vivo* testing.[74,86,94,116]

For this study, we had two hypotheses. Firstly (1), that activation of the DLPS would reduce TLI during airflow testing compared to no activation. Secondly (2), we hypothesised that activation of the DLPS would prevent dynamic collapse of the rima glottis during airflow testing.

6.3 Materials and Methods

6.3.1 Specimen preparation

Larynges and a segment of attached trachea from 10 Standardbred and Thoroughbred horses aged 3-11 years were collected and frozen at -20^{0}C. The larynges were then thawed at room temperature for 24 hours immediately prior to testing. Once the larynges were confirmed to have grossly normal anatomy, the thyroid, cricoid, epiglottic, arytenoid cartilages, 2 tracheal rings and all intrinsic muscles were retained for testing, with all other soft tissue removed. The left cricoarytenoideus dorsalis (CAD) muscle was transected immediately caudal to its insertion on the muscular process of the arytenoid cartilage prior to testing.

6.3.2 Suture placement

For each larynx, the right side was fixed in a maximally abducted position for all of the testing.[91,116] As previously reported, for the left sided LP, #2 Fiberwire suture (Arthrex™) was passed using a trocar point half circle needle through the cricoid cartilage 1.5 cm lateral to the midline and 1.5 cm from the caudal edge.[91] For the arytenoid attachment a FASTak®II (AR-1324HF, Arthrex™) suture anchor was placed in the arytenoid 10 mm cranial to the insertion of the CAD muscle as previously described.[91,113] The suture was then threaded through the eyelet of the anchor from medial to lateral such that the two thread ends were in a relatively lateral position creating a single suture loop.

6.3.3 Vacuum Chamber Model

A unidirectional vacuum system based on the *in vitro* models reported by Cheetham *et al. 2008* and others, was used for testing (Figure 1).[74,86,94]

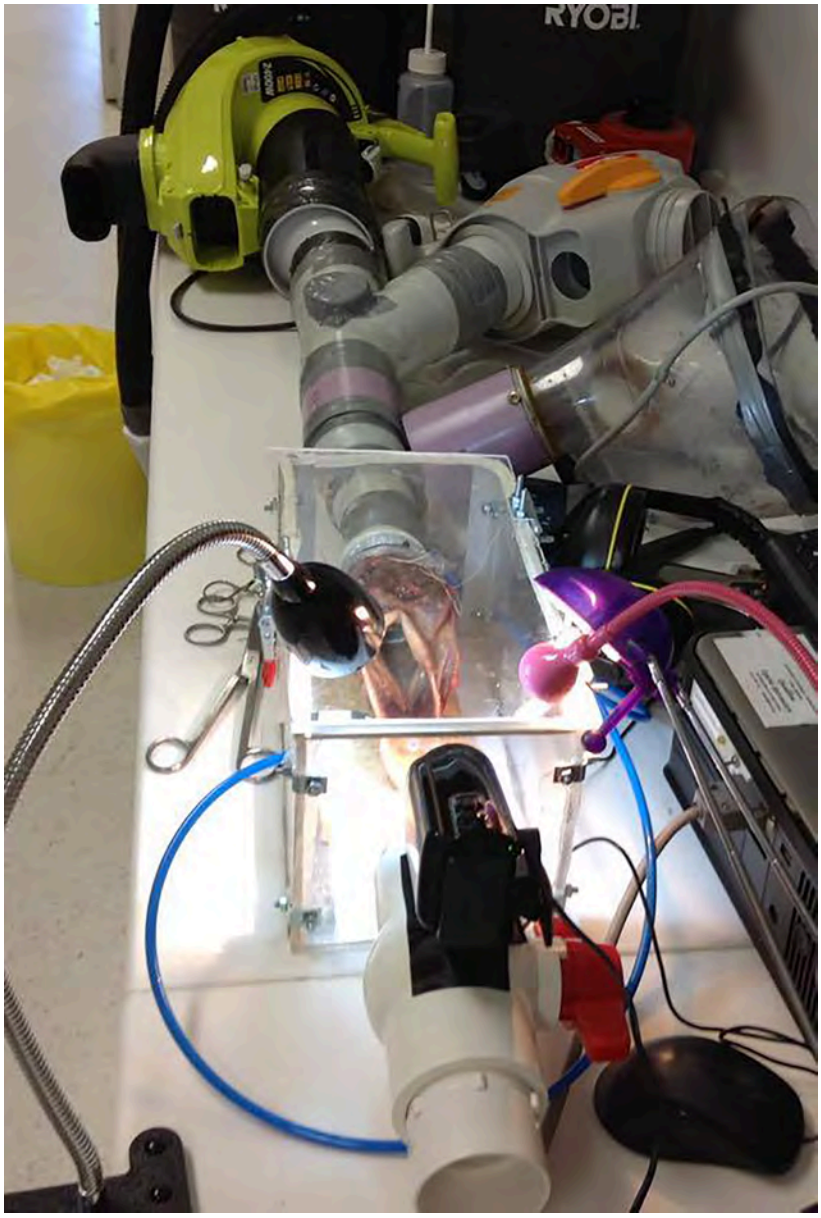

Figure 1. Model set-up for airflow testing showing the plastic box with the test larynx inside connected to the vacuum cleaners. The camera is mounted with lights focused on the larynx to ensure a clear image with no shadows for easy evaluation.

To achieve a variable flow rate, two vacuum / blowers (Ryobi 2400W 230-240V 50 Hz), set on vacuum mode controlled using a variable autotransformer (Variac, Powertech SRV-5), attached to a ergospirometer (Quadflow Equine Spirometry, QF, RobacScience Pty Ltd) were connected via a 50mm PVC pipe to the testing chamber. The chamber was

15x15x30cm with a 50mm hole at both ends of the chamber to allow for insertion of the 50mm pipe attached to the vacuum system and a variable inlet valve at the other end. A hole was drilled into the dorsal surface of the 50mm PVC pipe and into the flow chamber cranial to the position of the larynx to insert the catheters of the differential pressure transducer (Digitron Differential Pressure Transducer). Each larynx was affixed to the PVC pipe using cable ties to ensure an airtight seal between the tracheal rings and the pipe. The epiglottis was secured to a piece of balsawood on the bottom of the flow chamber using an 18-gauge needle. A 20x20mm white cardboard square was affixed to the balsawood in the same plane as the *rima glottidis* as a reference and in frame of a digital camera (HC-V250 high definition digital camera, Panasonic, Osaka, Japan), which was mounted above the inlet valve and focused on the *rima glottidis.*

Subsequently, the left arytenoid was maximally abducted using the Fiberwire equal to the right arytenoid and the flow and inlet valve altered to achieve a target flow rate of 55L/sec and a pre-laryngeal pressure (representative of pharyngeal pressure) of 12mmHg. This was achieved by adjusting the inlet valve and the voltage regulator to achieve a flow and pressure similar to previously reported *in vitro* and *in vivo* studies.[74,86,94,116,118] The Fiberwire was then completely loosened to allow for testing to commence. Each larynx was tested under static airflow conditions for a single five second period to obtain data during sequential testing as outlined below. Airflow data (flow in L/s and translaryngeal differential pressure in mmHg) were recorded for the entire five seconds of testing and the middle three seconds averaged for analysis. Translaryngeal impedance was calculated by dividing the translaryngeal pressure differential by the flow to produce the TLI (mmHg/L/s).

6.3.4 Left to Right Quotient Angle (LRQ) and Cross Sectional Area (CSA)

A still image was obtained after securing the construct at the desired position and before ("before") the airflow test was initiated. Subsequently, a still image was obtained at 2.5 seconds into ("during") each five second test period. These images were used to calculate the resultant LRQ and CSA for both before and during each test. The change in LRQ (ΔLRQ) and CSA (ΔCSA) was calculated by subtracting the before and during values as a means to detect any dynamic airway collapse of the *rima glottidis* caused by airflow testing. The LRQ and CSA was measured and calculated as previously described from each photograph by one observer using a soft-ware package (Image J, Bethesda, USA).[51,110] In short, for LRQ a line was drawn from the ventral to dorsal aspect of the *rima glottidis* and extended by one third to give a point that was used to measure the left (L°) and right (R°) arytenoid angles. The LRQ was then calculated by dividing L° from R°. Similarly, using Image J the 20x20 mm white square was used to set the scale of the program to each photograph and subsequently, the CSA of the *rima glottidis* was determined in mm^2.

6.3.5 Phase 1 testing – suture shortening

For Phase 1 testing, the ends of the suture at the middle of the lateral aspect of the cricoid cartilage were attached to the arms of a Vernier Calliper to allow for progressive controlled construct shortening at 3mm increments by opening the calliper arms as previously reported.[119] Airflow testing, as outlined previously, was completed sequentially for each of the following steps; 0 mm (suture loop tight and secured but no change in arytenoid position from resting) and then successive 3 mm shortening to a maximum of 30 mm of shortening (a total of 11 steps per larynx).

6.3.6 Phase 2 testing – DLPS activation

Immediately following Phase 1 testing of each larynx, the suture was tied routinely with a surgeon's knot and 4 single throws to fix the left arytenoid at an approximate "before" LRQ of 0.5 (i.e. left arytenoid at approximately half of the right sided maximal abduction). This starting LRQ was selected based on previous *in vitro* analysis that demonstrated that maximal activation of the DLPS was able to cause an increase in LRQ from 0.7 to 0.97 (an effective range of 0.27).[119] As a result, if the target LRQ at maximal abduction to significantly reduce TLI was to be approximately 0.75 (approximately a CSA of 88%) then the starting LRQ was estimated to be approximately 0.48 (i.e. 0.75-0.27=0.48). To allow for a margin of error given that this testing involved negative airway pressures and the previous *in vitro* study that generated the LRQ effective range of 0.27 was in resting larynges, we selected a starting LRQ for testing of 0.5.

Subsequently, the prototype was completed by inserting the DLPS into position between the completed suture loop. An inactivated baseline position (approximate LRQ of 0.5) and then two levels of activation were achieved by variably injecting saline into the catheter port. The level of activation was selected based on previous mechanical testing results; inactivated (0psi), moderate activation (25psi) and maximal activation (50psi).[115] As previously reported, the injection pressure was measured using a pressure syringe (Blue Diamond™ Merit Medical).[115] If a pressure of 50psi was not achieved the maximal pressure was recorded and the reason for failure to achieve 50psi also recorded.

6.3.7 Statistical Analysis

Data is reported as mean ± standard error of the mean (SEM). Normality was assessed with a Shapiro-Wilk test. Comparison of normally distributed data (impedance before and after testing) was made using a paired *t*-test. Changes in variables of interest (TLI, ΔCSA,

ΔLRQ) between baseline and at subsequent degrees of shortening and DLPS activation were compared using a repeated-measure analysis of variance (ANOVA). A Tukey *post-hoc* test was used when appropriate. Generalised estimating equations (GEE) or simple linear regressions were used to model the effect of shortening or DLPS activation on CSA and LRQ with (after) or without (before) flow. Significance was defined as $p < 0.05$.

6.4 Results

All testing was completed and data recorded without complication. The baseline flow and pre-laryngeal pressure at maximal bilateral arytenoid abduction (an LRQ of 1.0) prior to testing was 56.30 (+/-0.1) L/sec and 11.90 (+/-0.03) mmHg respectively.

6.4.1 Phase 1 testing

Progressive construct shortening from 0mm (0.60±0.11mmHg/L/sec) to 6mm (0.30±0.07mmHg/L/sec) had a significant effect on reducing TLI ($p=0.001$) (Figure 2). Further shortening to 9 mm (0.23±0.05mmHg/L/sec) or more caused additional smaller but non-significant reductions in TLI ($p>0.05$).

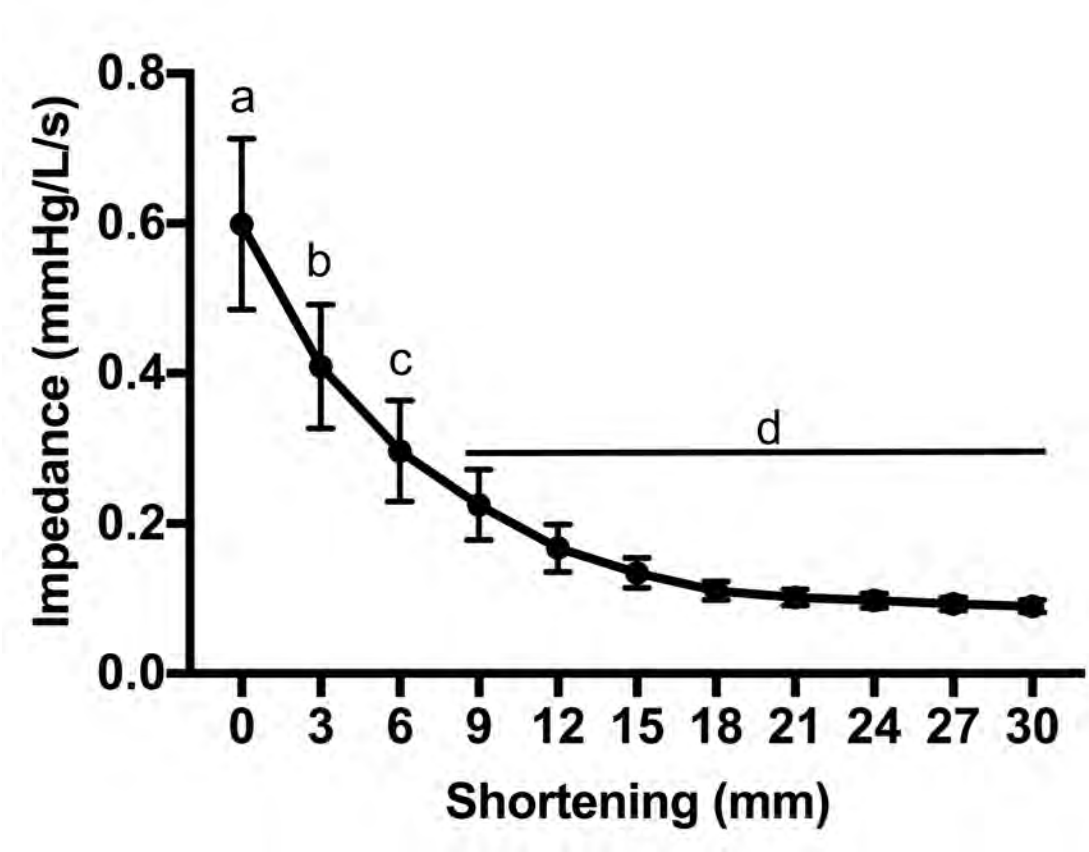

Figure 2. Change in TLI related to construct shortening in progressive 3 mm steps. Letters denote statistical significance.

Similarly, the ΔLRQ was significant ($p<0.001$) from 0mm (0.25±0.03) to 6mm (0.15±0.02) but stopped being significant with further shortening ($p>0.05$). The ΔCSA was significant from 0mm (721.98±57.89mm^2) to 15mm (350.88±35.73mm^2) but stopped being significant with further shortening ($p<0.001$) (Figure 3). During testing vocal fold collapse was note to occur in 3/10 larynges at maximal shortening. At 12mm of suture shortening (equivalent to maximal DLPS activation) 6/10 larynges were noted to have vocal fold collapse.

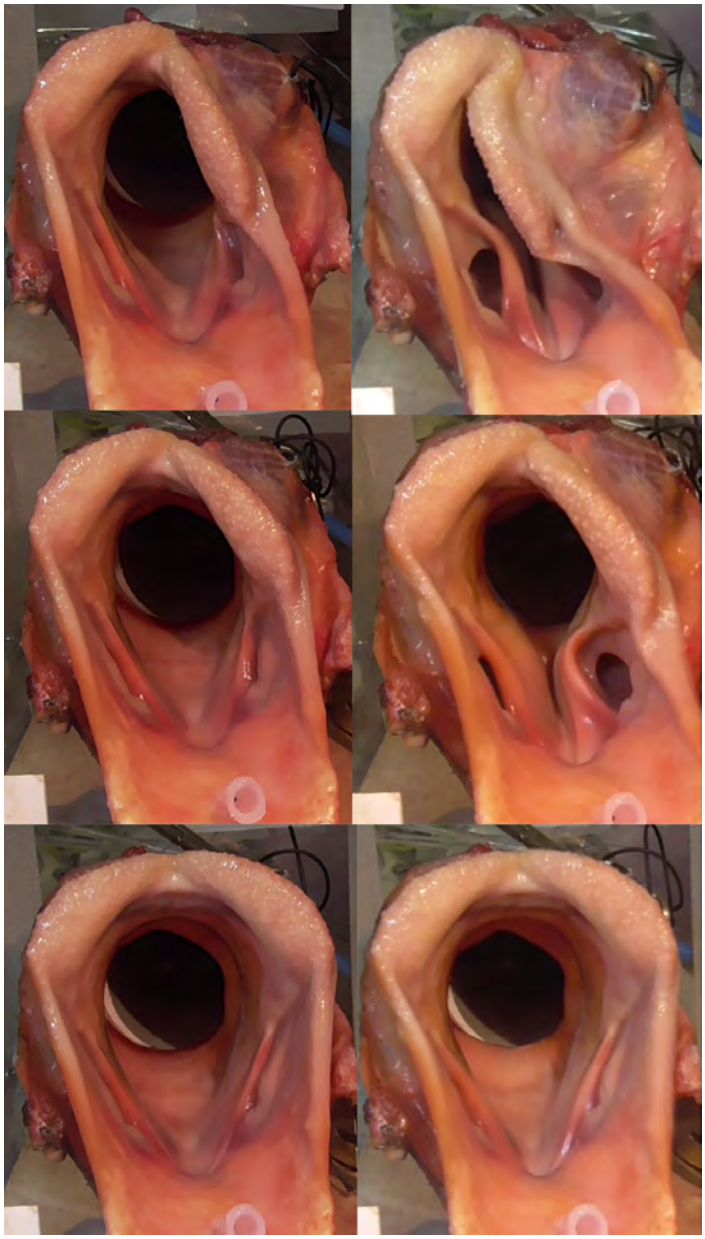

Figure 3. Representative images of rima glottis demonstrating ΔLRQ and ΔCSA between "before" (left) and "during (right) airflow tests for larynx #10 at 0,15,30mm (top, middle and bottom images respectively) of shortening. This represents a ΔLRQ and ΔCSA of 0.39 and 695.56mm^2, 0.09 and 382.64mm^2, 0 and 137.67mm^2, for 0,15 and 30mm of shortening respectively.

6.4.2 Phase 2 testing

Activation of the DLPS prototype caused a significant reduction in TLI from 0 psi (0.43±0.08mmHg/L/sec) to 25 psi (0.16±0.04mmHg/L/s)($p<0.001$) and a smaller non-

significant additional reduction in TLI (0.13±0.03mmHg/L/sec)($p>0.05$) with maximal activation (Figure 4).

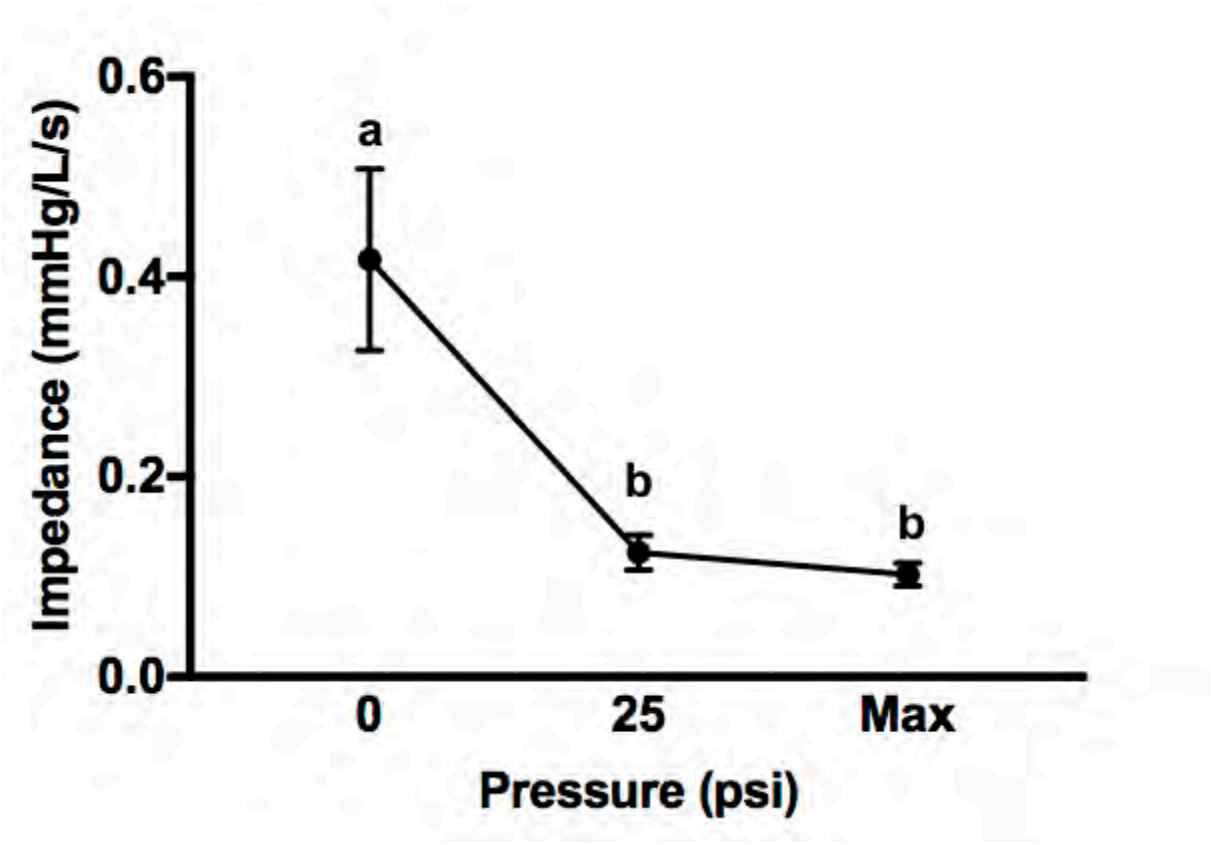

Figure 4. Change in TLI with DLPS activation. Letters denote differences in TLI of statistical significance ($p<0.05$).

A maximal activation of 31.3±0.96 psi was achieved during testing. Additional activation beyond this psi caused balloon prolapse from the DLPS and did not result in further change in arytenoid abduction. Using the results from Phase 1 of the study, maximum activation of the DLPS was found to have an effect on TLI reduction equivalent to approximately 12 mm of suture shortening.

There was significant reduction in ΔLRQ (dynamic arytenoid collapse) (Figure 6) at both levels of activation of the DLPS (25psi and max psi) compared to baseline (0 psi) (both $p<0.05$) (Figure 5).

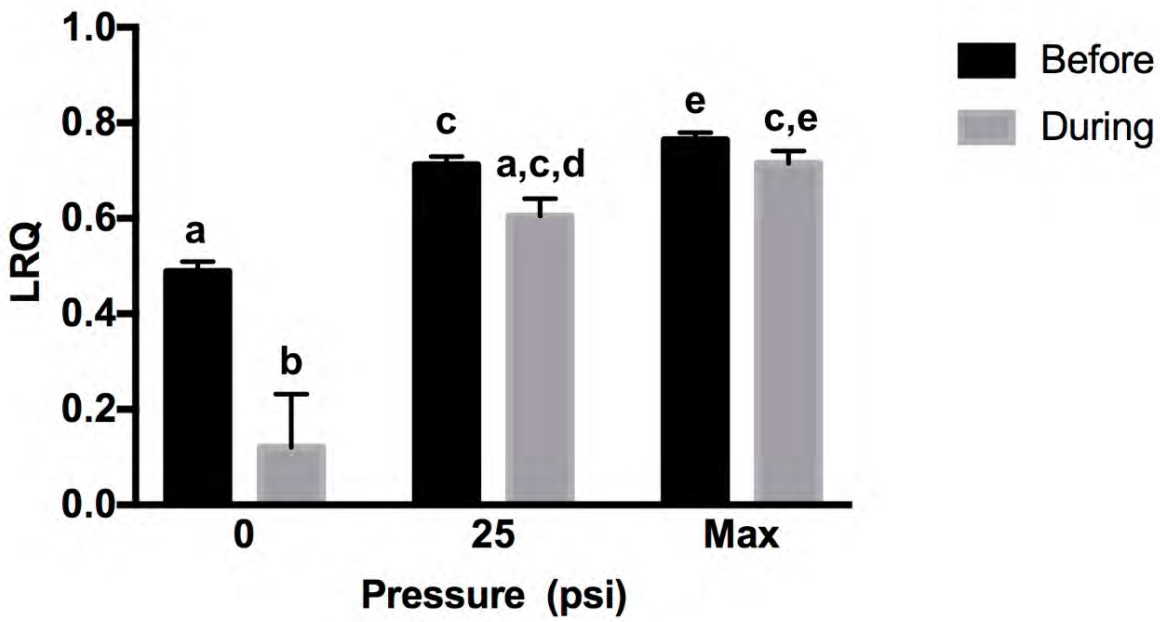

Figure 5. LRQ both before and during airflow testing with 3 levels of DLPS activation during airflow testing. ΔLRQ is the difference between the 2 at each level. Letters denote differences of statistical significance ($p<0.05$).

The ΔCSA was reduced by activation of the DLPS at both levels of activation compared to baseline but not significantly so (0, 25 and max psi, all $p>0.05$) (Figure 6). During testing vocal fold collapse was noted to occur in 5/10 larynges at maximal psi activation.

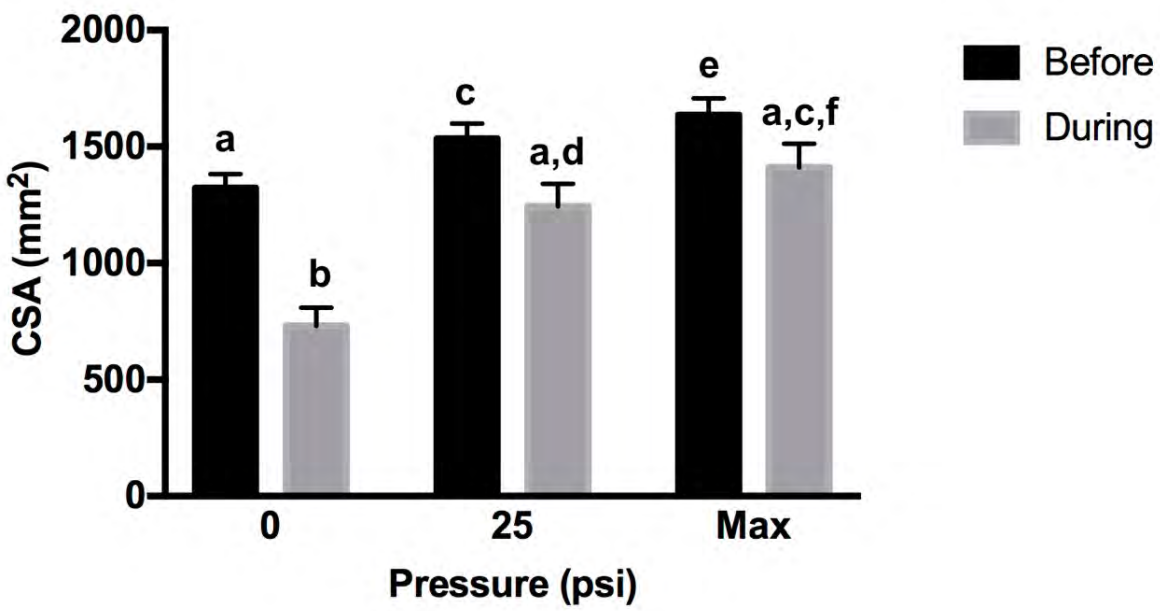

Figure 6. CSA both before and during airflow testing with 3 levels of DLPS activation during airflow testing. ΔCSA is the difference between the 2 at each level. Letters denote differences of statistical significance ($p<0.05$).

6.5 Discussion

The aim of the study presented here was to evaluate the DLPS prototype's ability to affect and maintain a reduction in TLI using a static airflow model. This study followed on from previous *in vitro* evaluations using a mechanical testing system that had found the prototype able to produce construct shortening of 7.11 mm whilst under a static 10 N load. Additionally, a separate study had found the prototype able to cause increases in LRQ in cadaveric larynges equivalent to 18.7 mm of construct shortening.[119] Based on the results of the study reported here we supported our first hypothesis by finding the DLPS was able to achieve a reduction in TLI equivalent to approximately 12 mm of construct shortening. We partly supported the second hypothesis in that the DLPS prevented dynamic collapse as determined by 25 psi and maximal activation preventing ΔLRQ but were not able to prevent ΔCSA during testing.

6.5.1 Translaryngeal impedance

The results from phase 1 of the study found that after 6 mm of construct shortening (tightening the suture) there was no additional reduction in the TLI. This is interesting as it indicates that relatively little shortening of the laryngoplasty suture construct can result in effective reductions in TLI that may be important for clinical success. Previous computational fluid mechanic modelling to measure the effects of different degrees of arytenoid abduction on upper airway characteristics found that a left arytenoid cartilage position resulting in 88% and 75% larynx cross-sectional areas resulted in 4.11 and 5.65% reductions in peak airflow.[21] Back calculating from our results, 88% and 75% would be equal 15-18mm (LRQ 0.7-0.75) and 6mm (LRQ 0.54) respectively (data not shown). Our results suggest that at 55L/sec flow, the minimum arytenoid abduction required to ameliorate negative effects on TLI would be an LRQ of 0.54 or a CSA of 75%. These finding may partially explain the results reported by Barakzai *et al* in 2009 that found post-operative performance after LP was not correlated with the degree of arytenoid abduction and suggested that horses with a post-operative grade 3 abduction (moderate degree of arytenoid abduction, approximately LRQ of 0.5-0.6) can race successfully.[111] However, the flow rates expected in maximally exercising Thoroughbreds have been reported to range from 65-100L/s and it is likely that the degree of shortening required to ameliorate any negative effects on TLI at these higher flow rates may be higher than the 6 mm reported in our study.[8,9,21,38,120,121] The flow rates used in our study are similar to those used in previous *in vitro* studies.[74,94,116] Unfortunately, it was not possible to evaluate the effect of higher flow rates due to system limitations with regards to effectively measuring flow. Further *in vitro* studies evaluating the effects on TLI at higher flow rates will be required to determine what effect this will have on the required degree of arytenoid abduction to ameliorate negative effects on TLI.

The results of phase 2 of the study demonstrate that the DLPS was able to reduce TLI when activated. The reduction in TLI was significant from 0 psi to 25 psi but maximal psi caused a smaller (non-significant) additional reduction. This may be due to the maximal psi being only 31.3 psi, as the target of 50 psi was not achieved due to the balloon segment of the DLPS prolapsing. This indicates that the target psi of 50 psi was too high when used *in vitro* likely because there is minimal resistance to inflation of the balloon in cadaveric larynges. *In vivo* the target activation psi will have to be determined but these *in vitro* findings suggest the maximum benefit of the DLPS prototype will be above 25 psi but below 50 psi, likely around 30 – 35 psi. Calculations (data not shown) using the data from this study revealed that no activation (0 psi) represented an LRQ of 0.49 and CSA of 66%, 25 psi activation represented and LRQ of 0.71 and CSA of 0.77% and maximal activation represented an LRQ of 0.76 and CSA of 0.82%. When the performance of the DLPS is compared to the phase one results of this study and the computational modelling of Rakesh *et al* 2008, the DLPS device was able to achieve increases in LRQ / CSA and resultant reductions in TLI that should be useful *in vivo*.

6.5.2 Dynamic laryngeal collapse

To evaluate our second hypothesis, we used the ΔLRQ and ΔCSA as a means of evaluating for any dynamic collapse produced by initiation of the airflow used in this vacuum system. Interestingly, the DLPS was able to prevent significant dynamic arytenoid collapse as measured by the ΔLRQ at both 25 psi and maximal psi but not when considered using ΔCSA. Review of the dynamic video recordings demonstrated this difference was mostly due to vocal fold collapse that influences ΔCSA measurements but not ΔLRQ. In phase 1 of the study at maximal shortening 3/10 larynges had vocal fold collapse compared to in phase 2 at maximal DLPS activation 5/10 larynges had vocal fold collapse. As a result of

this difference, we partly supported our second hypothesis depending on which measure of dynamic collapse was selected. A similar finding occurred in the phase 1 of this study with the ΔCSA being significant for 15 mm and the ΔLRQ for only 6 mm of shortening. Review of the images similarly found this was due to vocal fold collapse. Overall, these results support the clinical practise of performing a vocal cordectomy or ventriculectomy in conjunction with a LP to prevent dynamic collapse of the rima glottis due to vocal fold collapse. Similarly, clinical use of the DLPS will also benefit from a concurrent vocal cordectomy or ventriculectomy.

6.5.3 Model limitations

A major limitation of this model is that the airflow target of 55 L/s used, whilst in accordance with similar previous *in vitro* research, represents an airway pressure of horses exercising at approximately 100% VO2max.[118] However, airflows of up to 100 L/s have been recorded in maximally exercising horses.[38] Testing using these higher flow rates would be useful for further *in vitro* evaluations with regards to Thoroughbred racehorses. However, due to system limitations of only being able to measure a maximum flow of 60L/s and to allow for comparisons to previous research it was elected to select a target flow rate of 55 L/s.

Further limitations of the model are related to the use of cadaveric larynges, which is inherent to *in vitro* testing. Specifically, there is no muscular function in cadaveric larynges that may potentially have an effect on arytenoid abduction and TLI *in vivo*. The possible effect of drying of the larynges during sequential testing influencing the results was considered prior to testing. While there is no specific effect of drying known, each test was limited to a 5 second period in an effort to ameliorate any effect this may have had. During this research, each larynx underwent 14 sequential tests (11 shortening and 3 DLPS levels),

which was equal to a total of 70 seconds of active airflow during which no overt evidence of drying was noted. This period of airflow was similar to the 60 seconds used by Cheetham *et al*. 2008.[86] Finally, during our study the CAD muscle was transacted immediately caudal to its insertion on the muscular process. This was performed in an effort to minimise any potential effect the cadaveric CAD muscle may have on arytenoid position during the testing and ensure a free range of movement of the cricoarytenoideus joint. Clinically, surgeons may choose to transect the CAD muscle to improve access to the muscular process or to allow access to the cricoarytenoideus joint to facilitate fusion procedures.[74] For this experiment it was considered that transecting the CAD would minimise any potential effect the cadaveric CAD muscle may have between larynges, simulate complete laryngeal paralysis and as a result allow for the most accurate comparisons to be performed.

In conclusion, the prototype DLPS was able to cause reductions in TLI using a static airflow model. This reduction in TLI was approximately equal to 12 mm of suture shortening (tightening). The DLPS was able to prevent dynamic arytenoid collapse as measured by lack of change in the LRQ but did not prevent dynamic vocal fold collapse, resulting in an overall decrease in CSA during dynamic testing. Overall, the DLPS prototype evaluated using this *in vitro* airflow system was shown to be able to achieve and maintain arytenoid abduction that was beneficial to airflow. Based on these results further evaluation *in vivo* is supported.

PART IV: *IN VIVO* PROOF OF CONCEPT STUDY

Chapter 7: *In vivo* evaluation of a prototype dynamic laryngoplasty system (DLPS) in horses

Statement of authorship

Title of Paper	*In vivo* evaluation of a prototype dynamic laryngoplasty system (DLPS) in horses
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Principal Author

Name of Principal Author	Benjamin J. Ahern		
Contribution to the Paper	Designed experiments (80%) Performed experiments (100%) Wrote the paper (100%)		
Overall percentage (%)	90%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	20-6-17

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Andrew W. Van Eps		
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Contribution to the Paper	Designed experiments (10%) Critically reviewed paper (50%)		
Signature		Date	20/06/17

7.1 Abstract

Objectives- Evaluation of a novel dynamic laryngoplasty system (DLPS) in horses – a feasibility study.

Study Design- *In vivo* experimental.

Study units- Adult horses (n=3).

Methods- Three healthy Standardbred horses had a standing surgical procedure to induce complete laryngeal hemiplegia (transection of cricoarytenoideus dorsalis (CAD) tendon of insertion), which was subsequently treated using the DLPS. Activation of the DLPS was achieved using an injection port exiting through a skin stab (n=2) or using a subcutaneous injection port (n=1). Endoscopic examinations of the upper respiratory tract were performed pre-, intra- and at 7 days post-operatively. Left to right quotient (LRQ) ratios calculated during inactivated and activated states were obtained from still images of the rima glottis acquired at 7 days post-operatively. After these images were acquired the device was intentionally overinflated to evaluate for device failure. Post-mortem examinations were performed on day 7 to evaluate placement of the DLPS and to evaluate any complications or failures.

Results- No complications occurred in the 7-day post-operative period and the DLPS was effectively positioned in all 3 horses during a standing procedure. The LRQ at day 7 post-operatively could be varied from a resting position of 0.73 (+/-0.07) to a maximum of 1.01 (+/-0.06). At post-mortem, all DLPS components were functional with 2 not between the suture loop and the 3rd modified DLPS in the correct location.

Conclusions- Using the DLPS it was possible to alter the degree of left arytenoid abduction post-operatively in 3 adult horses with experimentally induced laryngeal hemiplegia. Minor modification of the device to restrain the suture loop within the device prevented failure caused by intentional over inflation.

Clinical Relevance- The DLPS may provide a useful alternative to traditional LP but requires further long term investigation.

7.2 Introduction

The dynamic laryngoplasty system (DLPS) has been developed with the overarching goal of being able to alter the degree of arytenoid abduction post-operatively in horses. With this goal in mind, the developed prototype has been evaluated *in vitro* in 3 steps. The first step was mechanical testing of the device demonstrating the device could cause functional shortening of a suture loop whilst under a static load likely to be seen *in vivo*.[115] The second step demonstrated the device was able to cause increased arytenoid abduction when activated.[119] The final third step using a static airflow model demonstrated the device was able to reduce translaryngeal impedance when activated.[119] Based on these *in vitro* findings a proof of concept *in vivo* study was proposed to evaluate DLPS deliverability and performance for the short term post-operative period.

The aims of this study were to 1) evaluate the surgical delivery of a DLPS in standing horses, 2) determine the ability of the device to alter arytenoid abduction via an injection port at 7 days post-operatively and 3) evaluate the devices' location at post-mortem examination for any errors in delivery or other types of failure.

We hypothesised that the DLPS 1) would allow for effective controllable alteration in arytenoid abduction at 7 days post-operatively, and 2) would not cause significant complications associated with its use. We elected to perform the surgery in a standing position, as previously reported, to allow for intra-operative assessment of arytenoid positioning during DLPS placement.[72]

7.3 Materials and Methods

Three adult Standardbred horses aged between 4-10 years old were enrolled in this study, which had institutional animal ethics approval (SVS/220/16). All horses' larynges palpated

normally and an endoscopic examination at rest revealed normal laryngeal anatomy and function (Havemeyer grade 1 or 2) with no evidence of gross tracheal contamination.

7.3.1 Surgical procedure

All horses were restrained in stocks, sedated (using detomidine hydrochloride 0.01mg/kg IV and butorphanol tartrate 0.01mg/kg IV), and prepared for standing surgery. A video endoscope was placed via the right nostril and attached to the rope halter in a position to maintain the larynx in view throughout the procedure. Subsequently, an approach to the left side of the larynx was performed as previously reported for a standing laryngoplasty technique.[72] In brief, a 10cm long subcutaneous line of local anaesthetic was injected immediately ventral to the left linguofacial vein and an incision made along this line. A combination of sharp and blunt dissection ventral to the vein was performed to allow access to the left dorsolateral aspect of the larynx. At this point the tendon of insertion of the left cricoarytenoideus (CAD) muscle was isolated and transected to induce complete left laryngeal dysfunction, which was confirmed using the video endoscope. The DLPS was then placed as follows: Using a trocar point half circle needle, #2 Fiberwire (Arthrex) suture was passed through the cricoid cartilage approximately 1.5cm lateral to the midline and 1.5cm from the caudal edge. For the arytenoid attachment a FASTak®II (AR-1324HF, Arthrex) suture anchor was placed in the muscular process of the arytenoid cartilage, 10mm cranial to the insertion of the CAD muscle as described previously (Figure 1A).[113] The balloon element of the DLPS was then placed in position between the resultant suture loop (Figure 1B). At surgery, the left arytenoid was placed at an estimated left to right quotient (LRQ) angle of 0.7 when the DLPS was in an inactivated state. In the first two horses the attached catheter was exited through a stab skin incision at the caudal aspect of the incision prior to routine closure in layers. For the final horse the DLPS was modified to

prevent suture prolapse (a PEEK pillar perpendicular to the suture direction connecting the PEEK discs) and the catheter was tunnelled subcutaneously (Figure 1C) and connected to an injection port (Vital-Port® titanium vascular access system, IP-S7110, Cook Medical) (Figures 1D and 1E) that was placed in a subcutaneous pocket via a 3cm skin incision on the neck (Figure 1F). The catheters and the subcutaneous injection port were used to activate the DLPS by injection of saline into the exposed port or via a needle inserted transcutaneously.

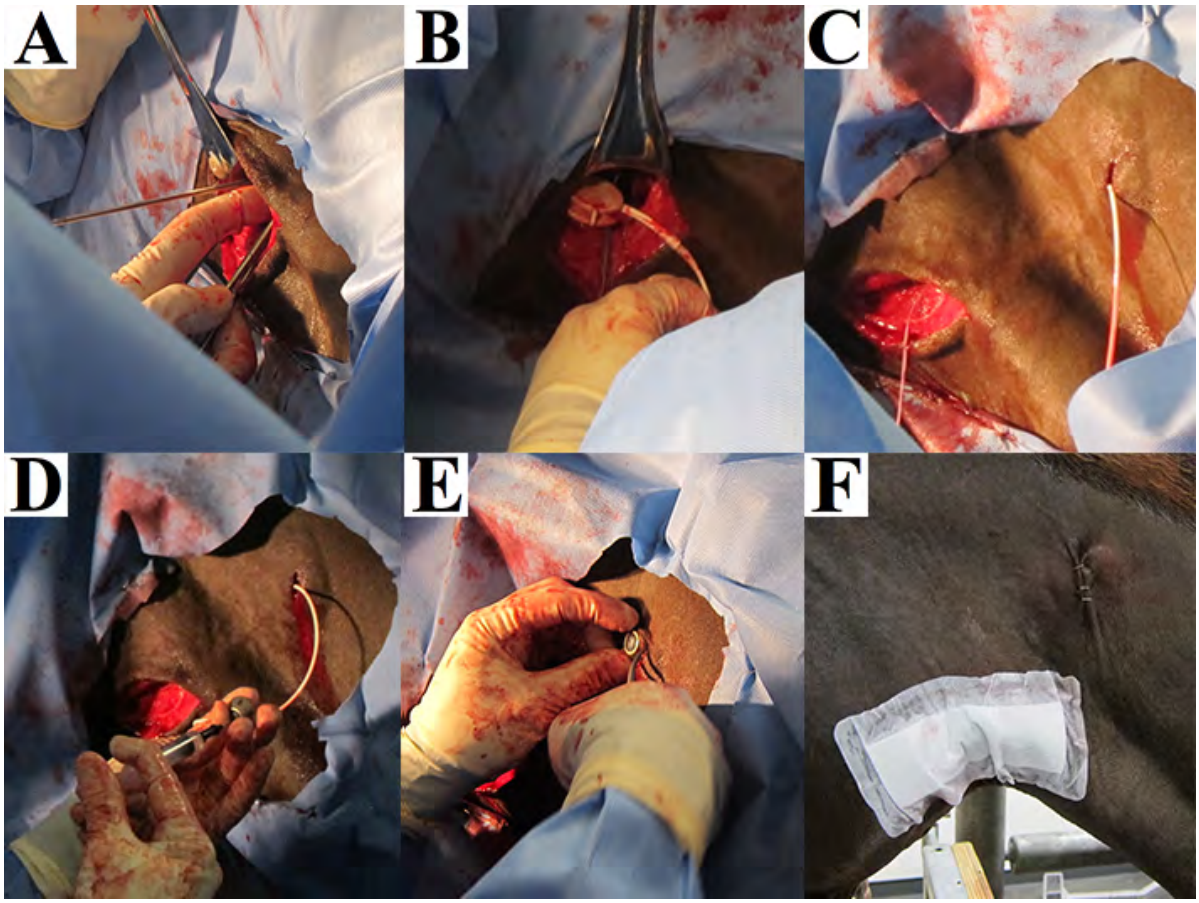


Figure 1. Intra-operative images of horse 3 demonstrating, A) arytenoid anchor placement, B) DLPS (with added pillar) being placed, C) catheter tunnelled subcutaneously, D) injection port being tested, E) subcutaneous injection port being placed and F) final post-operative appearance.

7.3.2 Post-operative care / examinations

All horses received procaine penicillin (22,000IU/kg) intramuscularly twice a day, gentamicin (8.8mg/kg) intravenously once a day and phenylbutazone (2.2mg/kg) orally once a day for 5 days post-operatively. Horses were fed from the ground in a hospital box for the duration of the study. At the completion of the study (7 days post-operatively) the horses were restrained in stocks and an endoscopic examination of the larynx and trachea performed via the right nostril. Any gross evidence of tracheal contamination was noted. Whilst observing the larynx endoscopically, the DLPS was activated by injection of saline (range 3-4ml) to assess the range of arytenoid abduction attainable and during maximal right arytenoid abduction. Once this was performed several times the DLPS was intentionally overinflated with 6 mls of saline. For the first two horses this activation was performed via the injection port exiting the surgical incision or in the final horse via the subcutaneous injection port using a needle inserted transcutaneously. Digital recordings were made of the endoscopic examinations at 7-days post-operatively and still images obtained pre-and post-inflation at points of maximal right arytenoid abduction. Subsequently, all horses were euthanized and the surgical site dissected to evaluate for any errors in device placement (i.e. if not located between the suture loop) or other types of failure (e.g. anchor movement, balloon rupture, prolapse or other construct failure).

7.3.3 Left to Right Quotient angle ratio (LRQ)

Arytenoid LRQ was measured and calculated as previously described from each still image by an experienced observer (B.A.) using Image J.[51,110] In short, using a digital image, acquired during maximal stimulated right arytenoid abduction, a line was drawn from the ventral to the dorsal aspect of the *rima glottidis* and then extended by one third to give a

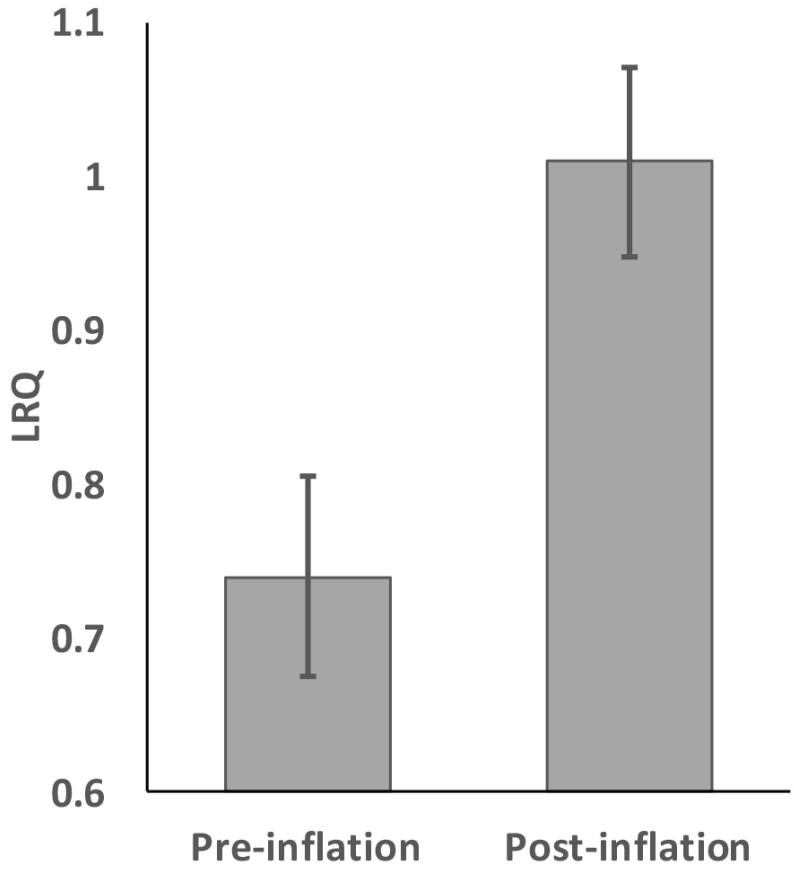
point that was used to measure the left (L^O) and right (R^O) arytenoid angles. The LRQ was then calculated from dividing L^O from R^O.

All data reported as mean +/- SD throughout unless otherwise stated.

7.4 Results

All surgical procedures were completed without complications, were of less than 45 minutes' duration and did not require additional doses of sedation. The DLPS device was readily positioned without complication.

Post-operatively all horses were clinically normal with no evidence of excessive pain or swelling of the surgical site and maintained a normal appetite for the duration of the study. During the post-operative period, no coughing or nasal discharge noted. Routine wound management was required for the 2 cases with catheters exiting the skin, with mild serous drainage being cleaned daily. The subcutaneous portal was visible and easily palpated. Injection into the portal was easily performed with routine restraint. The LRQ at day 7 post-operatively could be varied from a resting position of 0.73 (+/-0.07) to a maximum of 1.01 (+/-0.06) (Figure 2 and 3).



1843

1844 Figure 2. LRQ (mean +/- SD) at 7 days post-operatively pre-and post-inflation of the

1845 DLPS.

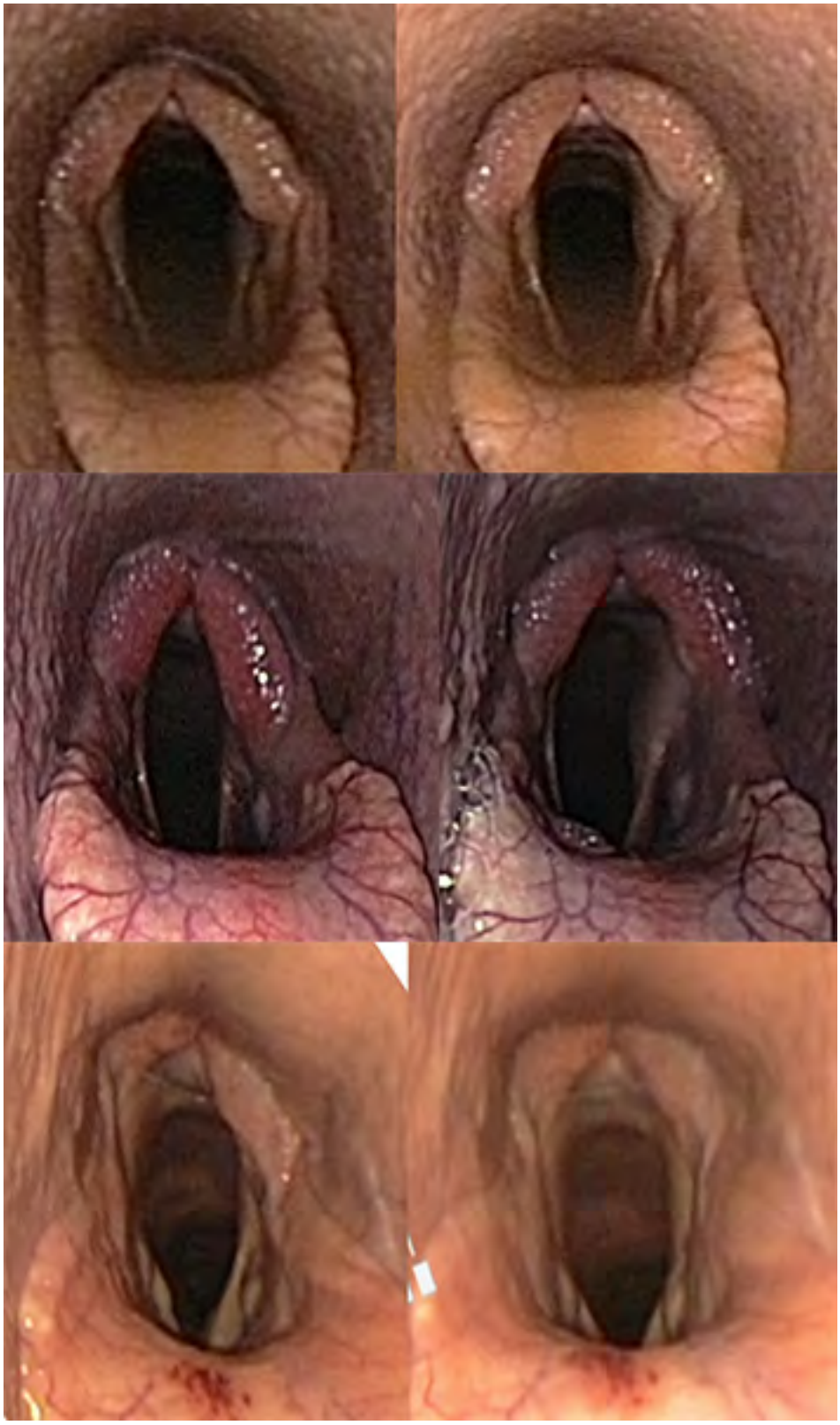


Figure 3. Endoscopic images of the three horses (top to bottom) at day 7 post-operatively pre (left) and post (right) inflation of the DLPS in each horse respectively.

Intentional over inflation of the balloon using 6 mls of saline caused a sudden loss of abduction in the first two horses. This loss of abduction did not occur in the third horse and in this horse additional injections of saline caused no apparent effect on the observed maximal arytenoid abduction. When the saline was removed in the third horse after over inflation, the arytenoid returned to the resting position. Endoscopic examinations of the trachea revealed no gross evidence of food aspiration in any case.

At post-mortem, for the first two horses, the device was displaced from the suture loop and sitting immediately adjacent and dorsal to the suture loop. The balloons were intact and the DLPS unit functional but out of position. For the third horse the DLPS was in place and activation of the device caused changes in arytenoid abduction as expected. Overall, the remainder of the construct (suture loop and anchor) were in place without evidence of failure in all three horses. There was a smooth lined tissue space at the surgical site with a small amount of serous fluid.

7.5 Discussion

We aimed to evaluate the feasibility of delivering a prototype DLPS in standing horses that was able to selectively alter arytenoid abduction at 7 days post-operatively. Based on the results from this limited number of horses we achieved “proof of concept” that post-operative selective alteration of arytenoid abduction was feasible using a prototype DLPS. Specifically, we supported our first hypotheses in that the DLPS allowed for effective controllable alteration in arytenoid abduction at 7 days post-operatively. Regarding our second hypothesis, no post-operative complications were identified in any of the three horses over the 7 days of the study. However, over inflation of the device in the first two horses led to displacement of the suture, leading to a failure to abduct the arytenoid cartilage on subsequent attempts. A post-mortem examination found the DLPS were not in

the correct position between the suture loop (displaced), which prompted a minor device modification for the third horse to prevent this displacement occurring. As a result of this modification and despite intentional over-inflation the 3rd device was in correct position and able to cause changes in arytenoid abduction. The balloon and PEEK components remained intact in all three cases.

This was a pilot study and clearly has the limitations of only a small number of horses and only a short survival period. This study suggests that the device is well tolerated and allows for variable adjustment of arytenoid positioning in the short term. Longer term evaluation of the device should investigate the effects of potential peri-prosthesis fibrosis or long term failure due to repetitive loading.

From a mechanical point of view, recent *in vitro* evaluation using limited cyclical and ramp to failure testing suggests that the balloon element of the device should be able to withstand at least preliminary *in vivo* loading.[115] The remaining element of the DLPS is made from polyetheretherketone (PEEK) which is commonly used in medical devices as it is safe, easily manufactured and impervious to moisture and degradation.[122] Finally, the catheter and injection port (used in horse three) is a subcutaneous portal system already used routinely in human medicine.[123] As a result, it is expected that these components will be very robust. The balloon component, whilst used in medical settings in an indwelling location, is the relative point of weakness in the DLPS prototype due to the cyclical functionality required. This component will warrant evaluation regarding longer term *in vivo* performance as it has not been used previously in a similar setting that we are aware of. At post-mortem, the DLPS in the first two horses had been displaced from between the suture loops by intentional over inflation of the balloon. This was done in an effort to

determine if the balloon would rupture or how the device would fail. For the third horse the PEEK element of the DLPS had a pillar of PEEK added at both peripheries of the device at 180^{o} to each other and perpendicular to the suture loop, to prevent the suture from displacing from within the confines of the device. At post-mortem, this device was still in location and functioning. This minor modification to prevent accidental over inflation should be used in all future similar devices.

How the DLPS will perform in an exercising pressurised larynx is also unknown at this time. Previous *in vitro* testing found the construct to be very stiff and 9 out of 10 constructs did not fail during ramp loading to 100N.[115] Furthermore, an *in vitro* evaluation of the DLPS using a static airflow model showed the DLPS could achieve an increase in and maintain arytenoid abduction that resulted in a significant reduction in translaryngeal impedance during airflows of 55L/second.[124] As a result of the previous *in vitro* evaluations and this *in vivo* proof of concept pilot the DLPS warrants further longer term evaluation likely using an exercising model.

Delivery of the DLPS *in vivo* during a standing procedure was readily achieved in this pilot study. Surgical familiarity with the DLPS and technique is essential prior to attempting effective delivery and will be directly related to surgical outcome. Standing surgical delivery was considered to be desirable to allow for the most accurate selection of the desired degree of arytenoid abduction during normal respiration. However, the DLPS could be used under general anaesthesia if desired and the authors have performed the procedure using cadaveric horses in lateral position as for a more traditional laryngoplasty performed under general anaesthesia.

There are a number of potential uses for the device including correction of disappointing post-operative abduction, or to correct more gradual post-operative abduction loss.

Secondly, the device might be used to selectively increase the degree of arytenoid abduction during periods of exercise and improve the athletic performance of the horse as a result. Finally, the device might also be used for research applications related to the effects of differing degrees of arytenoid abduction on airflow and lower airway contamination, using each horse as its own control.

The clinical significance of being able to alter the degree of arytenoid abduction post-operatively with regards to the incidence of longer-term complications and general performance are unknown. However, the results of this research achieved our aim of delivering the DLPS in sedated standing horses and allowed for effective alteration in the degree of arytenoid abduction at 7 days post-operatively. Based on the results of this study further longer term evaluation of the DLPS and exercise studies are warranted.

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PART V: CONCLUSION

Chapter 8: General Discussion

At the outset of this thesis we set out to develop a laryngoplasty technique that was fundamentally different to the traditional laryngoplasty in that it could be altered post-operatively. We theorised this may be useful to equine surgeons to allow for increased arytenoid abduction as a minimum and additionally might allow for a dynamic or variable laryngoplasty construct that could be used to "select" the optimal degree of arytenoid abduction for an individual horse.

We were successful in developing a DLPS that was able to produce and maintain a variable degree of arytenoid abduction in the face of representative airflows *in vitro*. Furthermore, we validated the prototype during a proof of concept *in vivo* study and found the DLPS allowed for controlled variation of the arytenoid position at 7 days post-operatively. This is the first reported laryngoplasty device that can readily and effectively cause alteration in the degree of arytenoid abduction post-operatively. This prototype or one of similar concept might be clinically useful to improve the success rate for horses treated for dynamic laryngeal collapse caused by RLN. Numerous different prototypes were conceived and subsequently discarded due to a variety of reasons prior to development of the final prototype DLPS that was used for *in vitro* and *in vivo* testing. During the final stages of the *in vivo* testing, a small modification of the device was made to prevent accidental over inflation. It is possible that with further *in vivo* study, such as using an exercising model, further modifications may need to be made in an effort to improve the functionality of the device.

The portion of the device that is the weak point is the balloon component. There are a variety of materials that the balloon can be made from and if this element of the device is a problem in future research than it will have to be revised. That being said, the remainder of the prototype is extremely robust and unlikely to fail *in vivo*. As a result of the robustness

of the device when inactivated (no balloon element functioning), the inactivated position selected at surgery should be at the lowest end of what is considered to be the minimal degree of arytenoid abduction. Based on the above research this would represent a target of slightly over a resting LRQ of 0.5 or a CSA of 0.75%, which equates to approximately 6mm of effective construct shortening. This degree of arytenoid abduction ameliorated the negative effects on TLI using an *in vitro* static airflow model with flow rates of 55L/min, similar to those that would occur at approximately 100% VO_{2max}.[118] Horses maximally exercising will generate flow rates significantly higher than this (reported up to 100L/sec) and they will likely benefit from a higher degree of arytenoid abduction that will be possible using the DLPS post-operatively.[8,120,121] This should result in maximisation of airway function by specifically allowing it to be tailored to an individual horse.

The effectiveness of the developed DLPS to return airflow mechanics and exercise capacity to normal whilst reducing complications such as tracheal contamination is currently unknown. Future work will include evaluating the DLPS using an exercising model similar to the one used by Radcliffe et al. (2006) that compared horses in 3 phases. These phases were; normal laryngeal function, induced laryngeal hemiplegia and after treatment with laryngoplasty or arytenoidectomy. We intend to evaluate the prototype using horses completing an exercise test on a high-speed treadmill at or as close as possible to racing speeds and conditions. The horses will undergo testing at; 1) normal laryngeal function, 2) induced laryngeal dysfunction and then using 2 arms, after treatment using either a traditional laryngoplasty or a DLPS. Similar to the Radcliffe et al. (2006) study the rate of complications and ability to return horses with laryngeal collapse to normal will be evaluated. After this exercising evaluation and over a longer term, the horses will be monitored intermittently to evaluate for any effect on functionality caused by potential fibrosis.

In conclusion, we developed a prototype DLPS and evaluated it thoroughly *in vitro* prior to demonstrating the proof of concept of the device *in vivo*. We demonstrated the effective surgical deliverability of the DLPS in horses using a standing sedated approach. Using the DLPS device the degree of arytenoid abduction could be effectively altered post-operatively which may be useful to equine surgeons in the future.

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