Post laryngoplasty ultrasonography of the horse larynx

By

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Master of Science
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DECLARATION

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Extension No. 1

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KINDLY NOTE:

Should there be a change in the species or number of animal/s required, or the experimental procedure/s - please submit an amendment form to the UP Animal Ethics Committee for approval before commencing with the experiment

APPROVED	Date 10 November 2016
CHAIRMAN: UP Animal Ethics Committee	Signature [signature]

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KEY TERMS:

Equine, larynx, ultrasound

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LIST OF ABBREVIATIONS

AC	Arytenoid cartilage
ACH	Arytenoid chondritis
BH	Basihyoid bone
CAD	Cricoarytenoideus dorsalis muscle also *M. cricoarytenoideus dorsalis*
CAL	Cricoarytenoideus lateralis muscle also *M. cricoarytenoideus lateralis*
CC	Cricoid cartilage
CR	Cranial
CT	Computed tomography
EVC	Equis Veterinary Clinic
GA	General anaesthesia
ICC	Intra-class correlation coefficient
MRI	Magnetic resonance imaging
RG	*Rima glottidis*
RLN	Recurrent laryngeal neuropathy
SEH	Summerveld Equine Hospital
SHM	Sternohyoid muscle also *M. sternohyoideus*
TC	Thyroid cartilage
VF	Vocal fold

GLOSSARY

Arytenoid chondritis (ACH) - an infectious or inflammatory response with ongoing ulceration or granulation on the arytenoid cartilage.

Laryngoplasty - extra luminal placement of a prosthesis between the caudal aspect of the cricoid cartilage and the muscular process of the arytenoid cartilage to simulate the action of the cricoarytenoideus dorsalis (CAD) muscle, maintaining the cartilage in an abducted position (also known as a ‘tie-back’).

Laryngeal hemiplegia - paresis or paralysis of the left arytenoid cartilage and its associated intrinsic muscles. More correctly termed recurrent laryngeal neuropathy (commonly termed ‘roaring’ or ‘gone in the wind’).

Recurrent laryngeal neuropathy (RLN) - a performance limiting neuro-muscular condition typically affecting larger breed horses, such as Thoroughbreds resulting in paresis or paralysis of the intrinsic muscles of the larynx, nearly exclusively occurring on the left hand side.

Ventriculectomy - Surgical removal of the laryngeal saccule (ventricle)

Ventriculocordectomy - Surgical removal of the vocal cord and laryngeal saccule (ventricle)

SUMMARY

Post laryngoplasty ultrasonography of the horse larynx

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Laryngoplasty is commonly used to treat laryngeal hemiplegia in Thoroughbred racehorses and success and evaluation thereof has traditionally been determined using endoscopy with little emphasis on ultrasonography.

Laryngeal ultrasonography can easily be performed in the standing unsedated horse and is relatively inexpensive. Ultrasonography of the normal horse larynx has been described, as have a number of pathological conditions that occur in the laryngeal and pharyngeal area. Postsurgical ultrasonographic evaluation of laryngoplasty has limited coverage in the literature and is limited to individual cases. The aim of the investigation was to evaluate and validate the use of transcutaneous ultrasonography in horses following laryngoplasty, as well as improving our understanding of the healing process and detecting postsurgical complications.

Laryngeal hemiplegia is a relatively common condition and is of particular importance in Thoroughbred racehorses. Traditionally, postsurgical laryngoplasty management and success has been determined by, and monitored with, resting upper respiratory endoscopy. This modality is limited in that peri- and non-luminal laryngeal structures cannot be assessed.

A prospective case series of 10 Thoroughbred racehorses with left recurrent laryngeal hemiplegia was examined prior to, 3-10 days, 30-50 days and 6-12 months after laryngoplasty and ventriculectomy, using transcutaneous ultrasonography and endoscopy. Arytenoid abduction was graded endoscopically at each of the examination times using the Havemeyer system for presurgery and the Dixon system postsurgery. The following measurements were made ultrasonographically: *cricoarytenoideus lateralis* muscle widest diameter, circumference and cross-sectional area; the distance between the vocal folds in exhalation and inhalation, basihyoid depth, rostral thyroid cartilage depth, and distance between the basihyoid and thyroid cartilage was also measured. The ultrasonographically visible presence or absence of the laryngeal ventricles was determined as well as the degree of laryngeal movement as absent, present or intermediate. Repeated means ANOVA was conducted on the ultrasonographic measurements at the four examination times with significance set at $P < 0.05$. Descriptive analyses on the vocal fold and arytenoid cartilage movement and visibility of the ventricles was performed.

It was determined that the structures described in a normal laryngeal ultrasonographic examination could be seen postsurgically. Postsurgical complications of laryngoplasty in the extra-luminal structures were found in seven horses and included soft tissue swelling, seroma formation and one case of marked subcutaneous haemorrhage. In addition a luminal vocal fold abscess and vocal fold granuloma were detected.

It was possible to visualise the presence or absence of a ventricular opening ultrasonographically in 70 % of postsurgical horses. The presence of the laryngeal prosthesis suture material was possible to determine in the healing tissues when metallic clips were used, but visualization of other material proved difficult. The distance between the vocal folds in exhalation over the long term was significantly larger than presurgically (1.00 ± 0.24 cm *vs* 0.67 ± 0.11 cm). The presurgical exhalation distance was also significantly increased from the 30-50 days mean. In addition, the comparison of vocal fold inhalation and exhalation distances at presurgery, 3-10 days postsurgery, 30-50 days postsurgery and at 6-12 months were all significantly different. When compared over time, the mean presurgical inhalation and exhalation means (1.09 ± 0.32 cm and 0.78 ± 0.28 cm, respectively) were significantly smaller than the 6-12 month inhalation and exhalation means, the 3-10 day means were significantly smaller than the 6-12 month inhalation and exhalation means, and the 3-10 day means were significantly smaller than the 30-50 day over time with an increase in the distance as healing progressed. Vocal fold and arytenoid cartilage movement was easy to evaluate ultrasonographically and appeared to correlate to the endoscopic grading, although the latter was not measured.

Caudal basihyoid bone depth means in the medium term were significantly different between 3-10 days postsurgery (1.85 ± 0.28 cm) and 30-50 days postsurgery (1.64 ± 0.14 cm). Over the long term the presurgical mean (1.62 ± 0.24 cm) was significantly less than 3-10 days postsurgery mean (1.93 ± 32 cm) and the latter mean was significantly larger than the 30-50 days postsurgery mean (1.66 ± 0.13 cm). In addition the 3-10 days and 6-12 month postsurgery means were also significantly different (1.93 ± 0.24 cm *vs* 1.60 ± 0.14 cm). Rostral thyroid cartilage depth means in the medium term at 3-10 days postsurgery (3.67 ± 0.53 cm) were significantly larger than 30-50 days postsurgery (3.24 ± 0.48 cm). These differences were most likely as a result of tissue swelling related to the surgery because the laryngoplasty and ventriculectomy had no influence on these structures: the 6-12 month postsurgery values are very similar to the presurgical values. No significant differences in any of the measurements of the cricoarytenoideus lateralis muscle were found at any measurement.

Future studies need to be performed with a larger sample size to establish a baseline of vocal fold excursion distance measurement to enable them to be used as a grading system for ultrasonographically evaluating the success of a laryngoplasty in the post laryngoplasty /

ventriculectomy horse. Additional objective endoscopic measurements of vocal fold excursion with correlation to ultrasonographic measurements also need to be performed.

Ultrasound is an easily accessible tool for most practitioners and can be used for, and will allow, easy evaluation of the post laryngoplasty larynx in the horse in assessing both the success (based on abduction grade) of the procedure, monitoring healing and detecting complications early in order to institute treatment early.

CHAPTER ONE: INTRODUCTION

1.1 BACKGROUND

Laryngeal hemiplegia is a not uncommon condition affecting horses and is of particular importance in Thoroughbred racehorses where it can be a cause of poor performance [1-4]. Laryngoplasty, with or without ventriculocordectomy, is a standard treatment for laryngeal hemiplegia [5, 6].

Upper respiratory endoscopy is used to diagnose a number of disorders of the larynx and pharynx [4, 7] and remains essential for accurate diagnosis and treatment selection [8]. Resting endoscopy should, wherever possible, be combined with some form of dynamic endoscopy (treadmill or over-ground) to complete the diagnosis [9, 10] and ensure dynamic dysfunctions are not missed [7, 11]. It is not clear whether treadmill or over-ground endoscopy is better for diagnostic purposes [12]. Both have disadvantages due to the respective equipment required and the inability to standardise the tests [12].

It has been shown that sedation reduces the observer repeatability of upper respiratory tract examination and grading [13, 14], particularly endoscopic grading of the larynx [15]. Application of a nose twitch does not affect the evaluation of the larynx [14].

Traditionally, postsurgical laryngoplasty management and success has been determined by, and monitored with, upper respiratory endoscopy. Even though this is the gold standard for intraluminal evaluation [16], this modality is limited in that peri- and non-luminal laryngeal structures cannot be assessed by means of traditional endoscopy [17].

Radiography has some use in the evaluation of the larynx, particularly for sub-epiglottic masses, intermittent displacement of the soft palate, measurement of epiglottic length and the identification of mineralisation and pathology of the arytenoid cartilage (AC) and cricoid cartilage (CC) [18-20], but has the disadvantages of superimposition of the structures and limited soft tissue detail. Postsurgically, radiography of the larynx is only capable of highlighting mass lesions and the presence of mineralisation or gas [17].

Magnetic resonance imaging (MRI) and computed tomography (CT) of the larynx have been described in the horse [21, 22] and in the diagnosis of upper airway conditions [23]. MRI is also able to show good soft tissue detail [17, 24, 25]. In clinical practice, these procedures are often impractical because of the high cost, availability, need for general anaesthesia (GA) and the risk associated with GA [25]. Computed tomography has the advantage of not always requiring GA but shows less soft tissue definition than MRI [8, 26]. The use of CT in identifying cartilage conformation has been described and may have an influence on prosthetic choice and placement during surgical planning for a laryngoplasty [22]. The use of postsurgical MRI and CT would be limited by the cost, availability and further risk of a second or third GA.

Laryngeal ultrasonography can easily be performed in the standing unsedated horse and is relatively inexpensive [17]. Ultrasonography of the normal horse larynx has been described [27-30] and also has been used to describe and diagnose a number of conditions that occur within the laryngeal and pharyngeal areas in horses [8, 29-34].

1.2 PROBLEM STATEMENT

Postsurgical ultrasonographic evaluation of laryngoplasty has limited coverage in the literature. In humans, postsurgical ultrasonography has been used to monitor healing in throat surgery patients in order to detect complications such as laryngeal neuropathies [35], while in horses it has been shown that infection of the laryngoplasty prosthesis and/or suture material can be identified ultrasonographically [31], early in the condition and postsurgical management can be effectively planned. Damage to the extraluminal laryngeal structures, such as the oesophagus, a potential, although uncommon complication when performing a laryngoplasty, can be identified [36] ultrasonographically and managed.

1.3 AIMS OF RESEARCH

The specific aim of the investigation was to evaluate and validate the use of transcutaneous ultrasonography in horses following laryngoplasty. This includes improving our understanding of the healing process, detecting postsurgical complications and comparing these to endoscopic findings.

CHAPTER TWO: LITERATURE REVIEW

2.1 DETAILED LITERATURE DISCUSSION

Laryngeal hemiplegia is a condition affecting larger breeds of horses [37, 38], where deterioration of the nerve fibres results in hemiplegia, with partial or complete paralysis of the intrinsic muscles of the larynx. This condition most commonly occurs unilaterally and affects the left laryngeal musculature, resulting in an inability to abduct the left arytenoid cartilage in a normal manner. There is convincing evidence that approximately 15 % of cases of laryngeal hemiplegia are progressive [39].

2.1.1 Surgical procedures

Laryngoplasty, where a prosthesis is used to mimic the function of the atrophied cricoarytenoideus dorsalis (CAD) muscle and the remaining intrinsic muscles of the larynx, as well as ventriculectomy and cordectomy, alone and in combination, have been used to improve performance of horses with laryngeal hemiplegia [6, 40-43]. Laryngoplasty is the treatment of choice for horses where airway obstruction would negatively affect performance [42]. A small number of studies looking at the effectiveness of ventriculocordectomy have been documented [44].

Laryngoplasty aims to improve the performance of the horse while ventriculectomy on its own, although claimed to improve performance [45], seems to only reduce the noise associated with the condition and has not convincingly been proven to enhance performance [42]. Horses having laryngoplasty without a ventriculectomy are 4.9 times more likely to make a noise postsurgically than those that had either bilateral or ipsilateral ventriculectomy [46]. With ventriculocordectomy, noise reduction may be less effective in some instances and is less predictable [37]. Another study reports that neither laryngoplasty alone, or with ventriculectomy, completely eliminates noise, suggesting that there may be other factors involved [6]. In two studies, 41 % and 46 % (respectively) of post left-sided laryngoplasty, horses had right-sided vocal fold collapse post laryngoplasty, even when left-sided ventriculocordectomy had been performed [47, 48]. However, there appears to be no advantage to performing a concurrent right-sided ventriculectomy when performing a left-sided laryngoplasty (and left-sided ventriculectomy) [46, 47]. In draught breeds, ventriculocordectomy has been shown to be more successful at reducing respiratory noise in horses with laryngeal hemiplegia than ventriculectomy alone [38] and at improving their performance [49]. Bilateral cordectomy has been described as an effective technique for reducing respiratory noise in horses with laryngeal hemiplegia when combined with a unilateral or bilateral ventriculectomy where noise reduction is the primary objective [42, 45]. Bilateral ventriculocordectomy has been shown to be approximately 20 % more effective at noise reduction than bilateral ventriculectomy alone [38], with bilateral ventriculocordectomy reducing noise more than unilateral ventriculocordectomy [50]. Some authors suggest that, while the inclusion of the bilateral cordectomy was effective in reducing respiratory noise, it is unlikely to affect clinical success when combined with a laryngoplasty [40], while others consider it to improve performance for low grades of laryngeal paresis [44, 45]. Some authors have shown that unilateral cordectomy (when performing laryngoplasty) does reduce airway obstruction as effectively as a bilateral cordectomy [51] and thus improves performance [52]. However, it does not improve respiratory noise production [51].

In a review of surgical procedure options, one study recommends a laryngoplasty combined with ventriculocordectomy as the optimal way to improve performance and reduce respiratory noise, leaving the option of bilateral or unilateral ventriculocordectomy up to personal preference [41]. It has, however, also been suggested that performing a laryngoplasty or combination procedure makes no difference in postsurgical racing performance [6, 40]. Success of laryngoplasty appears to be more closely correlated with breed, age, patient choice and the definition of a successful outcome [40, 53, 54]. A ventriculocordectomy has the advantage over a laryngoplasty (with or without a ventriculocordectomy) in having fewer complications [38, 45, 52].

In a survey of European equine surgeons, approximately 50 % perform laryngoplasty only, and 50 % combine it with ventriculectomy, with cordectomy almost always being performed concurrently with the ventriculectomy [55] (there is no indication in the study as to whether these procedures are uni- or bilateral, leading to an assumption that they refer only to unilateral procedures). Only a few of the respondents used either a ventriculectomy or ventriculocordectomy alone, without the laryngoplasty, and typically in ”milder cases” [55] (no grading system was reported in this study but it is likely that these would be grade 2 or 3 paresis as opposed to complete paralysis). In a similar survey of American surgeons, 83 % recommended laryngoplasty with ventriculectomy as their primary choice of procedure with only 7 % performing laryngoplasty alone [56]. There is therefore some controversy as to what is the best technique and or combination of techniques.

2.1.1.1 *Postsurgical complications*

Laryngoplasty is associated with a number of postsurgical complications. Common complications post laryngoplasty are: surgical wound infection, failure of the prosthesis to maintain abduction (short- or long-term), coughing, prosthetic infection, and aspiration pneumonia [57-59]. Additionally, known complications of laryngoplasty include: dysphagia, incisional swelling, seroma, infection, sinus tract formation, aryepiglottic fold collapse, vocal fold collapse, pharyngeal lymphoid hyperplasia, and upper oesophageal incompetence [5, 6, 31, 40, 46, 48, 49, 53, 57, 59-62]. Laryngoplasty failure may be due to suture-induced pressure necrosis of the AC or the suture tearing through the muscular process of the AC [60]. Ventriculocordectomy may also result in the previously mentioned complications, but additional complications include: laryngeal swelling, granuloma formation, and arytenoid chondritis (ACH) [41, 45, 52]. Additionally, the use of laryngoplasty with ventriculocordectomy results in an increased risk of inflammatory airway disease and exercise-induced pulmonary haemorrhage during exercise in the long term [63].

2.1.2 Ultrasonography

Transcutaneous ultrasonography of the normal equine larynx has been well described, including appropriate acoustic windows [27] (Figure 2-1).

Figure 2-1: Schematic representation of the equine larynx and hyoid apparatus (cranial to the left) showing the 4 acoustic windows described by Chalmers *et al. (2006)*. A: Rostroventral window, B: Midventral window, C: Caudoventral window, D: Caudolateral window (for left and right) (Image: rendered by Philp Norton[1])

[1] Adapted from original image obtained from https://www.slideshare.net/dvmfun/equine-larynx, Dane Tatarniuk, downloaded 26 September 2016.

Transcutaneous ultrasonography in the horse allows assessment of the normal laryngeal non-luminal structures, acknowledging the usefulness of examining laryngeal anatomy and function [27]. The normal laryngeal anatomy that can be visualised includes the following [24, 27, 29, 32, 34, 64]:

Rostroventral window (“A” in Figure 2-1)*:*

The lingual process of the basihyoid bone (BH), body of the basihyoid bone, ceratohyoid bones, the tongue

Midventral window (“B” in Figure 2-1)*:*

Caudal aspect of the BH and cranial aspect of the thyroid cartilage (TC), thyrohyoid bone with the attachment of the thyrohyoid muscle

Caudoventral window (“C” in Figure 2-1)*:*

Vocal folds (VF), vocal process of the arytenoid cartilage, trachea. Vocal fold movement can be seen

Caudolateral window (“D” in Figure 2-1)*:*

The TC, CC and AC. The *M. cricoarytenoideus lateralis* (CAL) can be seen between the TC and AC. Arytenoid movement can be seen.

Ultrasonography in equines has proven a useful tool in detecting abaxial arytenoid cartilage lesions [27] such as ACH [33] which can be seen as thickening of, and irregular margins of, the cartilages [17]. The visualisation of the CAL muscles, AC, VF and their movement can be identified ultrasonographically [27]. Vocal fold movement is easily visible in real time ultrasonography as is movement of the AC [27, 29]. This allows the clinician without access to endoscopy to ultrasonographically evaluate the larynx and diagnose laryngeal hemiplegia and conditions such as ACH [33]. Diagnosis of left laryngeal hemiplegia can be made ultrasonographically by observing increased echogenicity of the left CAL muscle [27, 29, 30] when compared to the right due to the atrophy of this muscle [29, 67, 68]. This echogenic change can be detected sub-clinically allowing earlier screening than with the traditional dynamic endoscopic examination [69]. This is supported by the correlation of endoscopic grading (using the Havemeyer system [70]) in horses and humans with the histopathological changes to the CAL muscle [71, 72] as well as the strong relationship with both resting and exercising endoscopy and ultrasonographic appearance of the CAL muscle [69, 73]. Additionally ultrasonographically guided biopsy of the CAL muscle is described and well tolerated in horses [74]. Ultrasound is well described as a tool in the diagnosis of human non-laryngeal muscular disorders [75-77]. Resting and dynamic endoscopy have been suggested to be particularly poor at predicting long term occurrence or progression of laryngeal hemiplegia [78] which suggests that ultrasound examination may be a better predictor of laryngeal hemiplegia [66, 69], particularly in the case of early or subclinical disease [67]. In human medicine, endolaryngeal endoscopic ultrasonography has been described and is predicted to have important intra- and postoperative evaluation applications in patients to examine perilaryngeal structures such as tumour masses [65] while transoesophageal ultrasound in equines looking at the intrinsic muscles of the larynx has shown that this may be a better approach for examination of the CAD [66, 67].

Occlusion of the nares causes more forced respiration, enhancing the ability to evaluate VF movement [27]. This allows laryngeal ultrasonography to be utilised as a valid diagnostic tool for diagnosis of laryngeal hemiplegia [29, 67]. Furthermore, horses with dorsal displacement of the soft palate have been shown to have ventral displacement of the caudal portion of the BH using laryngeal ultrasonography, although the reason for this is unclear [32]. Dynamic laryngeal collapse may be predicted [79] while laryngeal dysplasia can be accurately diagnosed ultrasonographically [24].

It is clear from the information above that the laryngeal abnormalities that can be visualized endoscopically can often be more comprehensively characterised using ultrasonography [17]. Therefore, laryngeal ultrasonography can be used as a sensitive and specific examination of the equine larynx, even allowing the examiner to determine AC movement. The grading of the arytenoid deficit can be difficult, however, due to the limited ability to visualize the position of the AC and the subtleties in its movement [73].

In cases with unknown history of laryngoplasty prosthesis surgery, placement and/or laryngeal advancement or the presence of suture material can potentially be detected on laryngeal ultrasonography [17]. A number of different prosthetic materials are used in the laryngoplasty procedure. These include, but are not limited to: nonabsorbable braided ethylene terephthalate, nonabsorbable monofilament nylon, nonabsorbable double stranded polyester, lycra, stainless steel, multi-strand ultra-high molecular weight polyethylene (Fiberwire[2]) and may or may not use a corkscrew anchor and/or suture button made of titanium [6, 57, 80-83]. Ultrasonographic visualisation of suture material has been described in dog and cat gastropexy sites postsurgery [84] and dog enterectomy and enterotomy sites [85]. In humans, Fiberwire is visible in postsurgical Achilles tendons [86] as well as rotator cuff tear repairs where the authors suggest that the presence of these materials may alter the ultrasonographic appearance and interfere with interpretation of the image [87]. In equine abdominal incisions, suture material is visible as small hyperechoic regions with distal shadowing in the *linea alba* ultrasonographically [88]. Similarly, in suture-

[2] Arthrex, Florida, United States of America

repaired equine tendons, the suture material is visible as hyperechoic foci with strong acoustic shadowing ultrasonographically [89].

CHAPTER THREE: RESEARCH HYPOTHESES AND OBJECTIVES

3.1 HYPOTHESES

There are six proposed hypotheses.

3.1.1 Hypothesis 1

Post laryngoplasty ultrasonographic examination is possible and the laryngeal structures can be seen.

3.1.2 Hypothesis 2

The ultrasonographic windows described by previous authors are valid post laryngoplasty.

3.1.3 Hypothesis 3

It is possible to differentiate a laryngoplasty versus a laryngoplasty plus ventriculectomy (or ventriculocordectomy) when examined on postsurgical ultrasonography.

3.1.4 Hypothesis 4

It is possible to detect postsurgical complications of laryngoplasty in the extra- and peri-luminal structures, if present.

3.1.5 Hypothesis 5

Upper respiratory endoscopy can be correlated to ultrasonography of the structures visible within the laryngeal lumen:

- Vocal folds
- Ventricles or lack thereof
- Arytenoid cartilages

3.1.6 Hypothesis 6

Extra- and peri-luminal structures can be better visualised ultrasonographically than endoscopically

CHAPTER FOUR: METHOD AND MATERIALS

4.1 EXPERIMENTAL ANIMALS

Ten Thoroughbred racehorses between ages 2 to 5 years (mean age 3.2 years) were used in this study. Horses diagnosed with left laryngeal hemiplegia that were due to undergo a laryngoplasty with or without a ventriculectomy or ventriculocordectomy were considered suitable for this study. Horses were selected from two equine practices in South Africa: Summerveld Equine Hospital (SEH) and Equis Veterinary Clinics (EVC) both located in Summerveld, KwaZulu-Natal. Both practices agreed to allow patients to be used for this study (Appendix 1). The owner or agent was contacted and given the relevant information regarding the study and signed consent was obtained (Appendix 2) for the horse to be examined ultrasonographically, endoscopically and to be sedated if necessary. Any results of the study would be given to the owner or agent if requested.

Patients were assigned a sequential study number LV 1-10 as they entered the study. The letters LV stand for the procedure performed: laryngoplasty plus ventriculectomy. Horses that were due to undergo a laryngoplasty plus ventriculocordectomy would be assigned the study number LC and a number as they entered the study to make up a separate group.

Should there be sufficient horses showing complications to create a normal and complications group these horses would be re-assigned to a separate group on ultrasonographic identification of complications (LVC). Should there be insufficient numbers to warrant separate groups the horses with complications will remain in the main grouping of horses.

At the point of surgery, the surgeon was allowed to perform a laryngoplasty or combination laryngoplasty and ventriculectomy or ventriculocordectomy to their preference and to what was clinically most appropriate to the case.

Sample group sample size comparing dependent means was calculated using the equation [90]:

$$n = \frac{\left(z_\alpha + z_\beta\right)^2}{\left(\frac{\delta}{\sigma}\right)^2}$$

Where:

$z_\alpha = 1.96$ (α = 0.05)

$z_\beta = 0.84$ (a power of 80 %)

$\left(\frac{\delta}{\sigma}\right)^2 = (0.9)^2$ (ability to detect a 0.9 difference in the means)

This results in a sample size of n = 10.

4.2 HOUSING AND TRANSPORTATION

The horses were housed in the relevant equine clinic stables and were transported to and from these clinics by the transport organised by the owners or agents of the owners.

4.3 FEEDING

The horses were fed according to the protocol of any patient admitted to the relevant equine clinic.

4.4 EXPERIMENTAL PROCEDURES

4.4.1 Data

Data collected were initially written on the data collection sheets. The data were then transcribed and saved in Excel spreadsheets on a computer at Summerveld Equine Hospital, and a copy, on a flash disk, was kept at the author's home. Images collected were similarly saved on a computer at Summerveld Equine Hospital, and a copy, on a flash disk, was kept at the author's home.

Data were collected on at least three separate occasions. As far as possible a long term follow up examination was also included. Data collection was done as follows:

1. Ultrasonographic and endoscopic presurgical examination, performed 24-48 hours presurgery.
2. Ultrasonographic and endoscopic examination postsurgery, performed 3-10 days postsurgery (short term).
3. Ultrasonographic and endoscopic examination follow-up postsurgery, performed 30-50 days postsurgery (medium term).
4. A 6-12 months follow up ultrasonography and endoscopic examination was performed if possible (long term).

4.4.2 Restraint, sedation or anaesthesia

Since horses are routinely evaluated ultrasonographically without sedation and sedation may possibly affect potential findings, it was preferable to examine all horses without sedation. If additional restraint was required, a neck or lip twitch was used and was so noted in the patient's data sheet. Horses that were intractable, even with a lip or neck twitch, but suitable for the study, were sedated with 0.01 mg/kg detomidine (Domesedan[3]) given intravenously prior to ultrasonography, repeated if necessary, and so noted on the patient's data sheet.

Postsurgical medication protocol for each patient was standard for the relevant clinic and included prophylactic antibiotic and anti-inflammatory therapy.

4.4.3 Preparation

Handlers familiar with horses held the horses and the stable manager assisted to handle any intractable animals.

4.4.4 Pre-ultrasonographic procedure evaluation

A clinical evaluation was performed by the primary veterinarian in charge of the surgical case to ensure the horse's health and suitability for the planned surgery (Appendix 3).

The endoscope was always passed through the right nasal passage of the horse with a lip twitch for restraint, to allow passage of the endoscope safely. All horses' presurgical endoscopy grading results were recorded on the data sheet (Appendix 4). The Havemeyer (2004) [70, 91] grading system was used. A video recording of the endoscopic examination was made and labelled with the horse's study number. All videos were graded by the

[3] Zoetis, Kalamazoo, Michigan, USA

researcher, blinded, at one sitting at the end of the study. To ensure accurate grading, endoscopic videos were graded a second time in a separate sitting one week after the first grading by the author, again blinding the case number during the grading. Intra-observer variation was calculated to determine repeatability of grading of the videos.

4.4.5 Ultrasonographic procedure

The horse was led into the procedure room of each relevant clinic and allowed to stand in a natural position with normal head carriage. In some horses the placement of the larynx behind the mandibular ramus made visualisation difficult. In these cases the horse's head was turned horizontally away from the side of examination, allowing the ramus to move forward in relation to the larynx. Ultrasonography was performed using a linear array transducer (5-10 MHz 38 mm) (Sonosite Titan Ultrasonography system, Sonosite Inc. Washington, United States) with alcohol as the coupling agent. The four acoustic windows, as identified by Chalmers *et al.* (2006) [27] (Figure 2-1), were used. Images from each acoustic window were saved and all measurements made where appropriate at the time of acquisition. The structures of the larynx and associated structures were evaluated using the acoustic windows. For each window the relevant structures were marked as 'y' representing 'yes' for being visible on ultrasonographic examination or 'n' representing 'no' for not being visible.

For examination of movement, the movement of the relevant structure was graded as 'absent', 'intermediate' or 'present'. An intermediate grading was deemed to be the presence of partial movement or when it was difficult to determine movement. An assessment of the effectiveness of the laryngoplasty was made where appropriate. This assessment was done by measuring the distance between the VF at the level of the vocal process of the AC to determine if this has increased or not. The structures visible and their relevant positions were described and any pathological conditions were noted (Appendices 5 and 6 for presurgical, 3-10 days, 30-50 days postsurgical and, if possible, 6-12 month postsurgical ultrasonographic examinations). As far as possible the suture/prosthesis length from rostral to caudal (the apparent distance) was measured on the two postsurgical ultrasonographic examinations. All measurements were made during the ultrasonographic procedure.

The following standardised images were obtained for each case at each of the three examinations for each horse; one of each of the following images was taken. Three measurements were made on each image and the mean of the three measurements was determined:

Image 1

Rostroventral window:

Placement of the transducer (Figure 4-1 A) for this window ("A" in Figure 2-1) shows the body of the BH in transverse plane (Figure 4-1 B). The depth of the BH was measured from skin surface to ventral margin of the BH in centimetres just caudal to the lingual process of the BH.

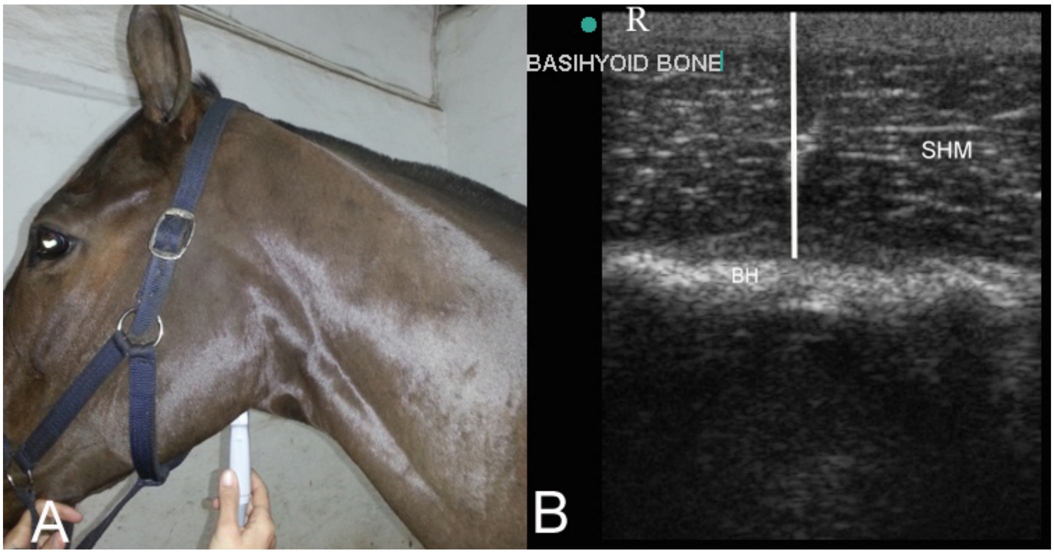

Figure 4-1 **A**: Transverse section imaging of the basihyoid bone. Positioning of the transducer for the rostroventral window. (Image: S. Miller). **B**: Transverse plane imaging the basihyoid bone of a normal horse. Right is to the left, ventral is at the top of the image, BH: basihyoid bone, SHM: sternohyoid muscle. The white line indicates the measurement from the basihyoid bone to the skin to be made in centimetres. (Image: S. Miller)

Image 2

Midventral window:

Placement of the transducer (Figure 4-2 A) for this window ("B" in Figure 2-1) shows the caudal aspect of the BH and cranial aspect of the TC and the sternohyoid muscles (SHM) (Figure 4-2 B). The following measurements were made: depth of the caudal aspect of the BH to skin surface in centimetres, the depth of the rostral aspect of the TC to skin surface in centimetres and the distance between the caudal aspect of the BH and the cranial aspect of the TC in centimetres.

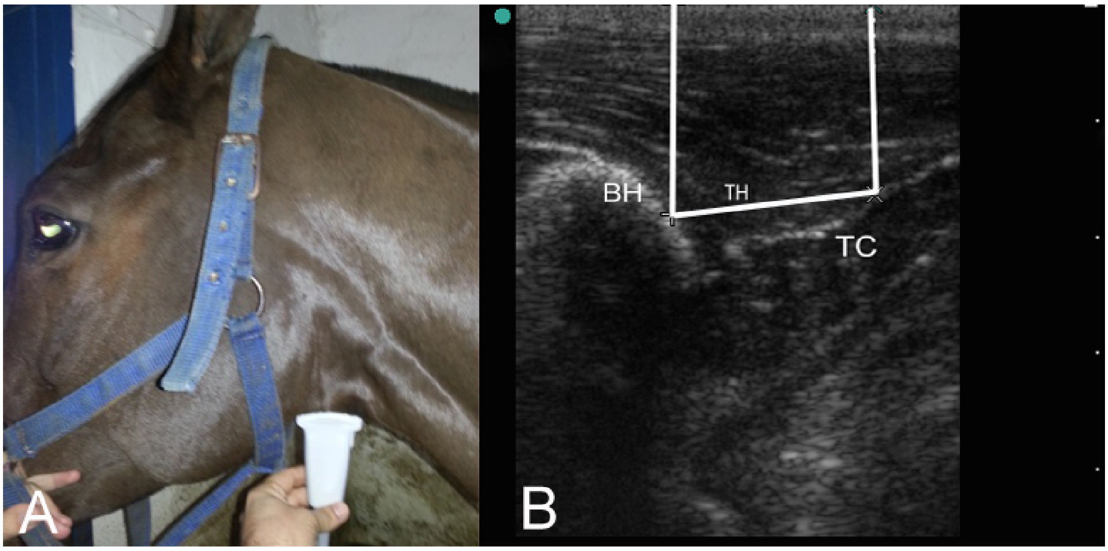

Figure 4-2 **A**: Longitudinal plane imaging of the caudal basihyoid bone and rostral thyroid cartilage. Positioning of the transducer for the midventral window. (Image: S. Miller). **B**: Sagittal image of caudal basihyoid bone and cranial thyroid cartilage of a normal horse. Cranial to the left, ventral at the top of the image. BH: basihyoid bone, TC: thyroid cartilage, TH: thyrohyoid muscles. The white lines indicates the measurements to be made in centimetres depth from skin to caudal BH, skin to cranial TC and distance between them. Thyrohyoid muscle insertion onto the abaxial thyroid cartilage. (Image: S. Miller)

Image 3

Caudoventral window:

Placement of the transducer (Figure 4-3 A) for this window ("C" in Figure 2-1) allows the VF, vocal process of the AC as well as VF movement to be visualised and imaged in the transverse plane (Figure 4-3 B). Vocal folds are seen as paired structures with the vocal processes of the AC deep to their border. Vocal fold movement was scored as present, absent or intermediate (where there is partial movement or it is difficult to determine). The distance between the VFs was measured during inhalation and exhalation in centimetres at the level of the most axial aspect of the vocal process of the AC and the VF junction across the rima glottidis (RG). Air within the RG casts an acoustic shadow between the vocal folds. Images were obtained when the vocal folds were at the furthest and closest distances apart during inhalation and exhalation respectively.

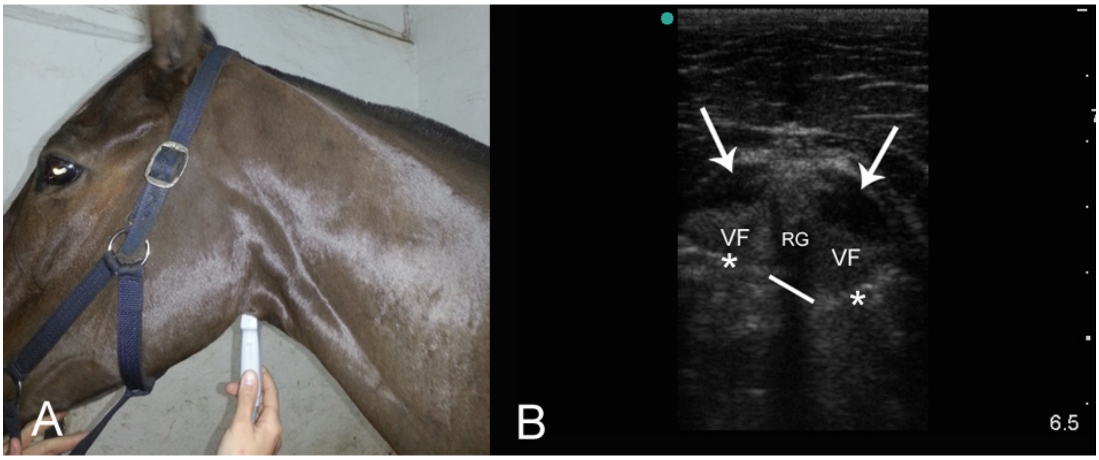

Figure 4-3 **A**: Transverse plane imaging of the vocal folds. Positioning of the transducer for the caudoventral window. (Image: S. Miller). **B**: Transverse plane at the level of the vocal folds of a normal horse. VF are the left and right vocal folds, with the laryngeal *rima glottidis* (RG) between them and the ventricles ventrally (white arrows). Right is to the left, ventral at the top of the image. The white line indicates the distance between the vocal folds made in centimetres at the level of the vocal process of the AC (asterisks). The vocal processes of the AC are visible as hyperechoic linear areas deep to the VF. (Image: S. Miller)

Image 4 and Image 5

Left and right caudolateral window - longitudinal

Placement of the transducer (Figure 4-4 A) allows the visualisation of the TC, CC and AC in this window ("D" in Figure 2-1). Between the TC and AC, the CAL muscle as well as arytenoid movement can be appreciated in real-time (Figure 4-4 B). The following measurements were made of the CAL muscle in centimetres: the widest diameter, the circumference, the cross sectional area. Left arytenoid abduction movement was scored as present, absent or intermediate (where there is partial movement or it is difficult to determine). The left and right sides were measured in an identical fashion.

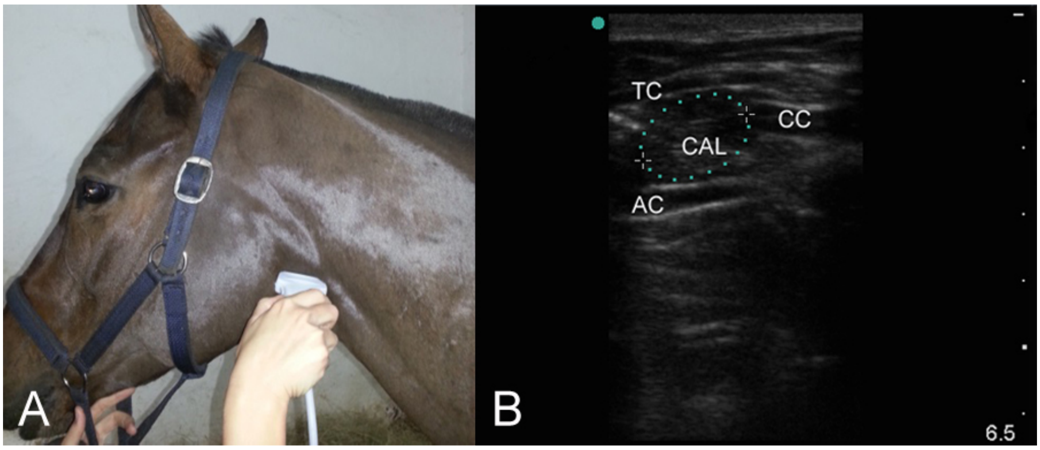

Figure 4-4 **A**: Longitudinal plane imaging of the thyroid, cricoid and arytenoid cartilages as well as the cricoarytenoideus lateralis muscle. Positioning of the transducer for the caudolateral window. Image shows positioning on the left; note that the positioning is the same on the right. (Image: S. Miller). **B**: Longitudinal plane imaging the thyroid (TC), cricoid (CC) and arytenoid cartilages (AC) and *cricoarytenoideus lateralis* muscle (CAL) in a normal horse. The dotted line represents the measurement of cross sectional area. Cranial is to the left and dorsal at the top of the image. (Image: S. Miller)

Image 6 and Image 7

Left and right caudolateral window - transverse

Placement of the transducer (Figure 4-5 A) for this window (“D” in Figure 2-1) allows the ACs and their movement (in real time) to be visualised (Figure 4-5 B). Left and right arytenoid abduction movement was scored as present, absent or intermediate (where there is partial movement or it is difficult to determine). The arytenoid cross sectional area and circumference was measured in centimetres and the margin of the AC described as smooth or irregular. The left and right sides were measured in an identical fashion.

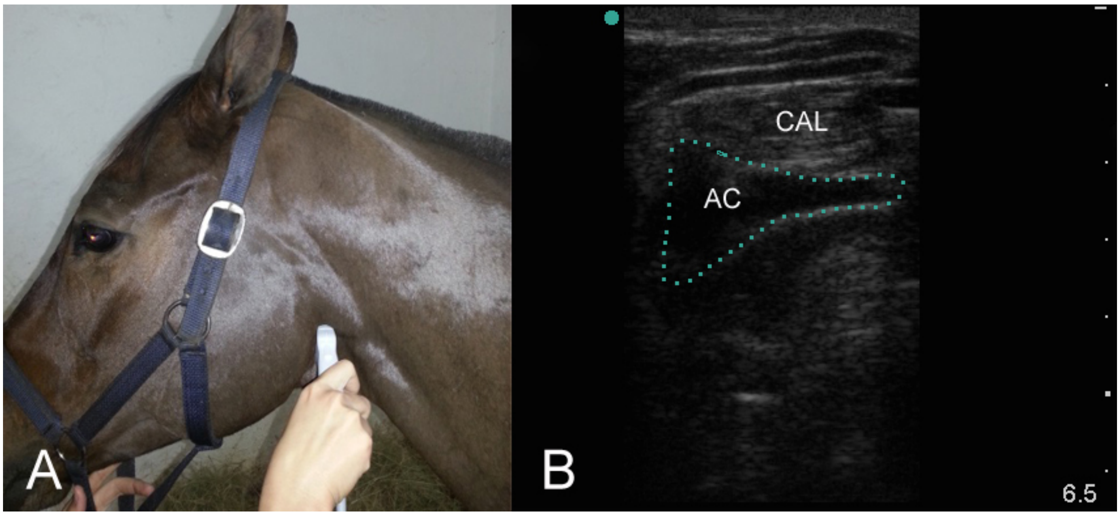

Figure 4-5 **A**: Transverse plane imaging of the Arytenoid cartilage. Positioning of the transducer for the caudolateral window. Image shows positioning on the left; note that the positioning is the same on the right. (Image: S. Miller). **B**: The typical trumpet shape of the arytenoid cartilage (AC) seen in this transverse image outlined by the dotted lines used to measure cross sectional area and circumference. Cricoarytenoideus lateralis muscle (CAL) visible above it. Cranial is to the left and dorsal at the top of the image. (Image: S. Miller)

4.4.6 Endoscopy procedure

Each horse underwent upper respiratory endoscopy to visualise the laryngeal structures at the same examination time as the ultrasonography (Portoscopes.com 12.9 mm portable video endoscope, Portoscope.com, Bradenton, Florida, USA). Each examination was recorded on video and evaluated in two sittings, one week apart, by the researcher (SMM) to determine intra-observer repeatability with the researcher was blinded to the case number. The endoscopic examination was performed at the same three stages as the ultrasonographic examination.

Presurgical grading of the larynx used the Havemeyer system as described in Table 4-1 [70].

Table 4-1: Havemeyer grading system for static endoscopic examination of the laryngeal structures.

Static Havemeyer grading (please circle appropriate grade) (Robinson 2004):	
Grade 1	All arytenoid cartilage movements are synchronous and symmetrical and full arytenoid cartilage abduction can be achieved and maintained.
Grade 2	Arytenoid cartilage movements are asynchronous and/or larynx is asymmetric at times but full arytenoid cartilage abduction can be achieved and maintained.
Grade 2.1	Transient asynchrony, flutter or delayed movements are seen
Grade 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.
Grade 3	Arytenoid cartilage movements are asynchronous and/or asymmetric. Full arytenoid cartilage abduction cannot be achieved and maintained
Grade 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.
Grade 3.2	Obvious arytenoid abductor deficit and arytenoid asymmetry. Full abduction is never achieved.
Grade 3.3	Marked but not total arytenoid abductor deficit and asymmetry with little arytenoid movement. Full abduction is never achieved.
Grade 4	Complete immobility of the arytenoid cartilage and vocal fold.

The postsurgical endoscopic assessment of the effectiveness of the laryngoplasty was estimated using the Dixon grading system [5] below by the researcher in one sitting for all horses at the end of the study. The postsurgical endoscopic grading was graded on a scale of 1-5 [6, 92, 93] as follows: Grade 1: close to or at maximal abduction contacting and depressing the pharyngeal wall (80°-90° to the sagittal plane), Grade 2: High degree of arytenoid abduction, contacting the pharyngeal wall (50°-80° to the sagittal plane), Grade 3: moderate abduction, not touching the pharyngeal wall (45° to the sagittal plane), Grade 4: slight degree of arytenoid abduction (slightly more than a resting position), Grade 5: no abduction (Figure 4-6).

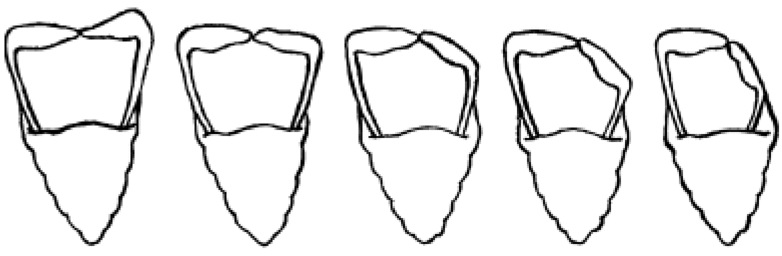

Figure 4-6. Schematic examples of postsurgical abduction grades (1-5) left to right. Image from Davidson *et al.* (2010) [5, 93]

Any additional pathological conditions were noted. The findings were recorded on the endoscopy form provided (Appendix 7).

For the purposes of this study only static (standing) endoscopic evaluation was utilised.

Complications were graded as mild, moderate or severe. Complications were graded as mild if they required no major additional treatment out of the norm postsurgically. Complications were considered moderate when they required additional medication and/or surgery not normal for the surgical procedure. Complications were considered severe when they resulted in failure of the laryngoplasty, which would include, but not limited to failure of the prosthesis, failure to abduct, infection of the prosthesis or surgical site, subcutaneous gas accumulation, deep tissue gas accumulation, draining tracts as a result of infection, and visualisation of fistulae leading to infected prosthesis or suture material.

4.4.7 Post-imaging procedure

The horses left the procedure room and were returned to their respective stables. They were returned to the clinician in charge of their case.

4.4.8 Observations

Observations of the position of the left AC of the larynx were noted to assess the effectiveness of the laryngoplasty using ultrasonography and endoscopy (see points 4.4.5 and 4.4.6 for description of measurements and grading). The additional structures visible on both the ultrasonographic and endoscopic images were noted and their positions recorded. Any pathological conditions at the time of examination were noted and described.

4.5 DATA ANALYSIS

The data were placed on a spreadsheet and analysed using SPSS[4]. All measurements of scale were assed for normality with a Shapiro-Wilks test as well as visually with stem and leaf plots [94]. The data were then analysed within a general linear model using Pillai's Trace [95] and Mauchly's Test of Sphericity to validate the use of repeated measures analysis of variance (ANOVA) with a Greenhouse-Geisser estimates of sphericity correction [96, 97] and Bonferroni correction [98] to control for Type I errors. Thereafter, due to the low case number in this study, the data were analysed using a nonparametric two-way repeated

[4] IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.

measures ANOVA, namely Friedman's test in pairwise fashion [99], which was used as the main basis of significance in the analysis. For significant results, a Wilcoxon signed rank test was performed [100]. Intra-observer reliability in the endoscopic grading was evaluated by means of intra-class correlation coefficient (ICC) determination using Cochran's Q test [101, 102]. One tailed repeated measures Students T-test [103] was used to examine the means of paired data over a time period. The significance was assessed at $P < 0.05$ for all tests performed.

4.6 RESEARCH EQUIPMENT

Ultrasonography: Sonosite Titan Ultrasonography system property of Summerveld Equine Hospital – Manufacturer: Sonosite Inc. 21818 30th Drive SE, Bothell, Washington 98021-3904, USA. ultrasound@sonosite.com

Endoscopy: Portoscopes.com 12.9 mm portable video endoscope property of Summerveld Equine Hospital – Manufacturer: Portoscopes.com, 7322 Manatee Ave West #265, Bradenton, FL., 34209, USA. info@1800endoscope.com

4.7 ETHICS

The animals were treated in a humane fashion consistent with the policies applicable to all patients admitted to the relevant equine clinics by professional handlers and veterinarians, and did not undergo any procedures that were considered untoward. The protocol for this study was evaluated by the Animal Ethics Committee of the University of Pretoria. Initial ethics approval was granted on 30 November 2015 for research until November 2016 (project number V115-15). An extension to this ethics approval was granted on 10 November 2016 to run until November 2017 by which time data collection had been completed.

CHAPTER FIVE: DATA ANALYSIS AND RESULTS

Of the ten horses in the study, there were two fillies, one colt and seven geldings (see Table 5-1). In total, nine horses came from Summerveld Equine Hospital and one from Equis Veterinary Clinic. No horse was sedated or twitched during the ultrasonographic examinations. All horses were subjected to a nose twitch for the endoscopic examination for ease of examination. In all ten horses a laryngoplasty with bilateral ventriculectomy was performed. In all nine horses referred by SEH, the same surgeon performed the surgery. The case referred by EVC (Horse LV8) was performed by a different surgeon; however an identical surgical technique was used. In all 9 SEH horses the laryngoplasty prosthetic was a 5 synthetic braided coated polyethylene terephthalate (Ethibond; Ethicon, United States of America). The case from EVC used monofilament nylon with metal crimp tube (Medium dog cruciate suture with crimp 80 lb breaking strength, Kyron labs, Johannesburg, South Africa).

Table 5-1: Gender and age of horses enrolled into the study at time of enrolment.

Horse Number	**LV1**	**LV2**	**LV3**	**LV4**	**LV5**	**LV6**	**LV7**	**LV8**	**LV9**	**LV10**	**MEAN**
Age at enrolment (years)	5	3	5	4	2	2	2	3	2	4	3.2
Gender	gelding	gelding	gelding	gelding	gelding	filly	filly	gelding	colt	gelding	

Cochran’s Q test returned an intra-class correlation coefficient of ICC = 0.857 (CI: 0.654:0.954). ICC values between 0.8-0.9 indicate good to excellent reliability of observer grading.

Examination at 3-10 days postsurgery in all of the horses examined showed a degree of purulent material ultrasonographically, particularly in the caudoventral window while visualising the VF. This material was typically of a viscous nature and could be appreciated at the laryngotomy opening during the examination. The appearance ultrasonographically was that of hyperechoic speckled material, mostly on the ventral surface of the *rima glottidis* but also on the edges of the vocal folds. In some instances gas was seen associated with the purulent material (Figure 5-1 A) while at other times the purulent material had no gas associated with it (Figure 5-1 B).

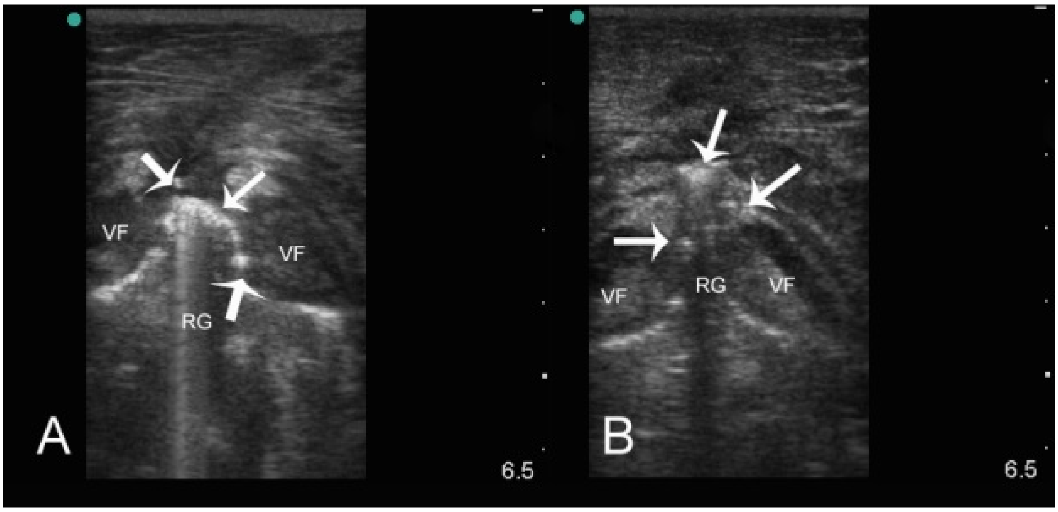

Figure 5-1: Purulent material on ultrasonographic examination at 3-10 days postsurgery. **A**: Purulent material (white arrows) with associated gas. Note the luminal ringdown artefacts created by the gas. This image is horse LV4. **B**: Purulent material (white arrows) without associated gas. This image is horse LV7. In both images A and B VF: vocal folds, RG: *rima glottidis*, ventral is to the top of the image, right is to the left. This is the caudoventral window – transverse plane. (Image: S. Miller)

5.1 HYPOTHESIS 1

Ultrasonographic evaluation of post laryngoplasty larynges in horses:

Post laryngoplasty ultrasonographic examination is possible and the laryngeal structures can be seen.

5.1.1 Postsurgical ultrasound examination, 3-10 days postsurgery

Generally, the normal structures of the larynx were visible in all ten horses. All left VF movements and AC movements were graded as absent and all right VF movement and AC movement were graded as present. In horse LV1, only one of the measurements of the CAL was possible because the CAL was difficult to image during this examination due to excessive swelling in the area. In horse LV7 the CAL was not detectable due to subcutaneous haematoma formation. The left AC was not imaged on this examination for LV7 for the same reason. For LV10 only one of the measurements of the CAL was possible. In three horses the CAL was not imaged. In one horse, the left AC was not imaged when viewed from the caudolateral window in the transverse plane. In summary, all normal structures were viewed in all examinations except the CAL which was only viewed in seven out of ten horses via the caudolateral window longitudinal approach, and nine out of ten times using the caudolateral window transverse approach. The caudoventral window may be influenced by the length and placement of the ventriculectomy surgical site. The author did not find this to be an obstacle to examination.

5.1.2 Postsurgical ultrasound examination, 30-50 days postsurgery

For all horses 30-50 days postsurgery the normal structures of the larynx were all visible. All left VF movements and AC movements were graded as absent and all right VF movements and AC movements were graded as present.

5.1.3 Postsurgical ultrasound examination, 6-12 months postsurgery

Six horses were examined for this period. All the normal structures of the larynx were visible. All left VF movements and AC movements were graded as absent and all right VF movements and AC movements were graded as present.

Thus from the above results it is clear that the normal structures of the larynx visible in the presurgical horse are readily identifiable in the postsurgical horse at 3-10 days, 30-50 days and 6-12 months postsurgery. A small number of normal structures were not visible in the 3-10 days postsurgery period. The CAL muscle was only imaged in seven out of ten horses (longitudinal view) and the AC was viewed nine out of ten times in the transverse view.

We can thus accept the hypothesis that the normal structures of the larynx can be seen in the postsurgical horse is correct.

5.2 HYPOTHESIS 2

Ultrasonographic evaluation of post laryngoplasty larynges in horses:

The ultrasonographic windows described by previous authors are valid post laryngoplasty

5.2.1 Postsurgical ultrasound examination, 3-10 days postsurgery

In three out of ten horses the CAL was not imaged in the caudolateral window in the longitudinal plane. In one examination the left AC was not imaged when viewed from the caudolateral window in the transverse plane.

In the caudolateral window transverse plane the left AC was imaged in nine out of the ten horses.

5.2.2 Postsurgical ultrasound examination, 30-50 days postsurgery

For all horses the normal structures of the larynx at 30-50 days postsurgery were visible in the applicable windows.

5.2.3 Postsurgical ultrasound examination, 6-12 months postsurgery

Six horses were examined for this period. For all horses the normal structures of the larynx at 6-12 months postsurgery were visible in the applicable windows. All left VF movements and AC movements were graded as absent and all right VF movements and AC movements were graded as present.

From the above results we can accept the hypothesis that the normal windows for examination for the equine larynx are valid in the postsurgical horse.

5.3 HYPOTHESIS 3

Laryngoplasty alone vs. Laryngoplasty plus ventriculectomy:

It is possible to differentiate a laryngoplasty versus a laryngoplasty plus ventriculectomy (or ventriculocordectomy) when examined on postsurgical ultrasonography

Since all horses in this clinical study underwent a bilateral ventriculectomy it was not possible to acquire comparative data for ventriculocordectomy. One could, however, determine if the ventricle openings were visible postsurgically or not.

For each postsurgical examination period, the set of values was evaluated. During presurgical evaluation, both left and right ventricle openings were visible in ten out of ten horses, represented by Figure 5-2 where the opening to the ventricles are defined as a hypoechoic area, ventro-lateral to the VFs. For postsurgical examination at 3-10 days, the left ventricle opening was visible in one out of ten horses and not visible in nine out of ten horses. The right ventricle opening was visible in two out of ten and not visible in eight out of ten horses. Figure 5-3 shows ventricle openings that are partially distorted and less easily evaluated at the 3-10 days examination. For 30-50 days postsurgery the left ventricle opening was intermediate in two out of ten horses and not visible in eight out of ten horses. Figure 5-4 shows intermediately visible ventricle openings with a more speckled appearance and a less clearly defined border. The right ventricle opening was visible in one out of ten horses, graded intermediate in two out of ten horses and not visible in seven out of ten horses. For the 6-12 months evaluation the left and right ventricle openings were visible in one out of six horses and not visible in five out of six horses. Figure 5-5 is representative of ventricle openings that are no longer visible, with the openings having reduced to a minimal hypoechoic line.

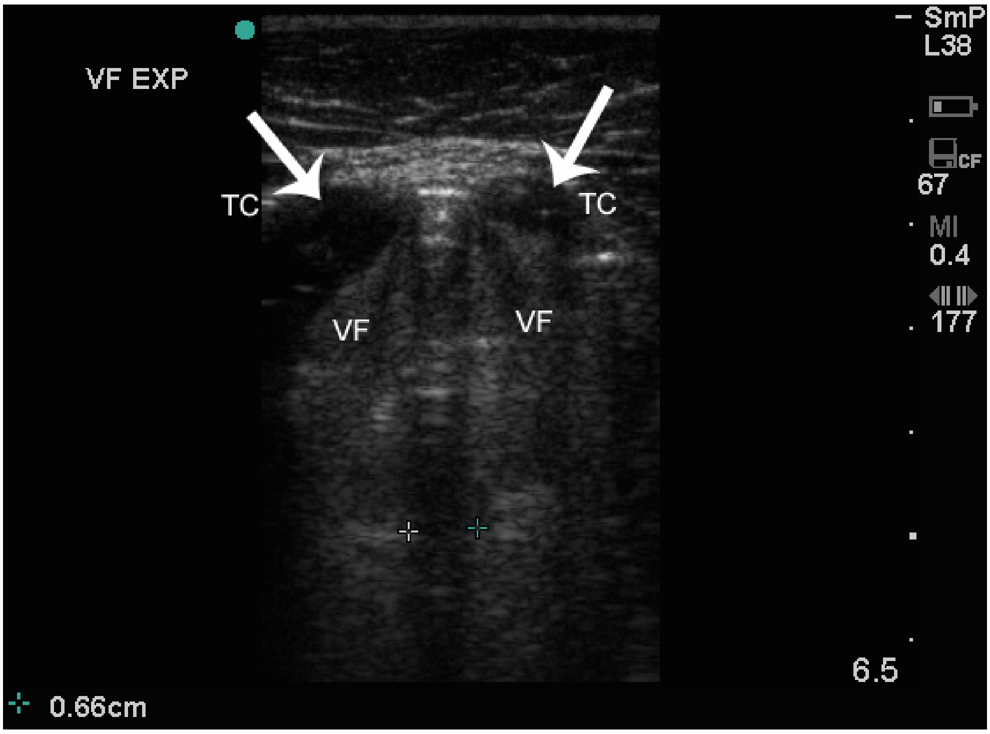

Figure 5-2: Presurgical ultrasound. Hypoechoic openings to the ventricle openings clearly visible (white arrows) in this image as hypoechoic spaces between the thyroid cartilage (TC) and the vocal folds (VF). Right is to the left of the image and skin/ventral is at the top. Plusses indicate the measurement of the expiratory vocal fold distance in cm. This is the caudoventral window – transverse plane. This image is from horse LV10. (Image: S. Miller)

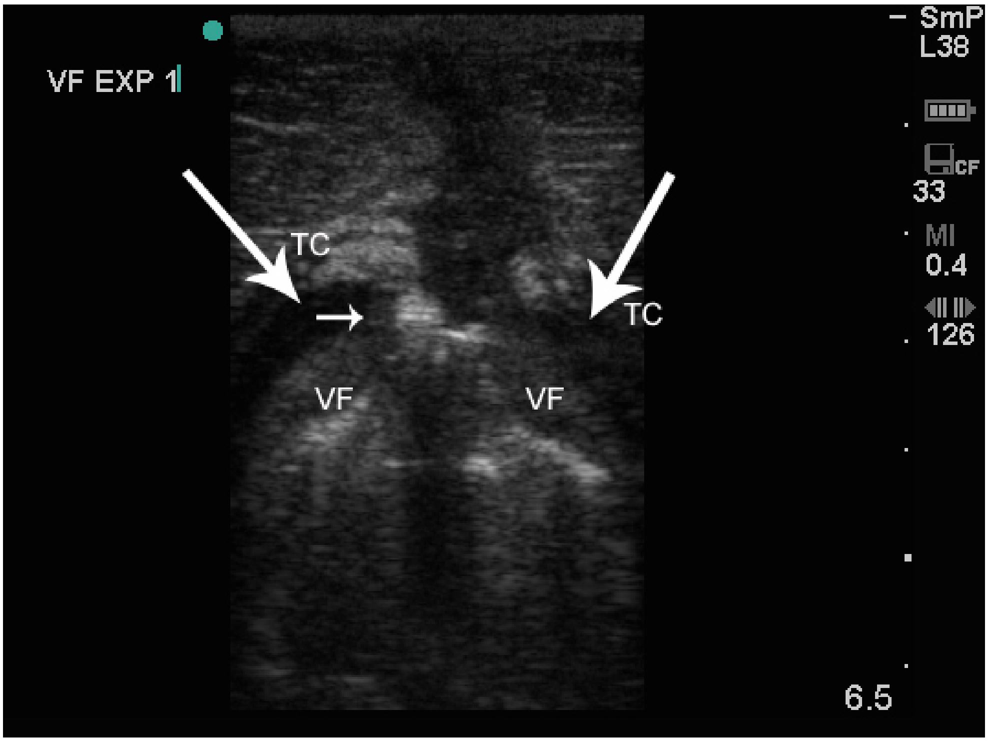

Figure 5-3: Postsurgical 3-10 days ultrasound. Smaller hypoechoic ventricle openings are not clearly visible in this image (large white arrows). The right ventricle entrance appears distorted and filled with hyperechoic speckled material (small arrow). TC: thyroid cartilage, VF: vocal fold. Right is to the left of the image and skin/ventral is at the top. This is the caudoventral window in transverse plane. This image is from horse LV10. (Image: S. Miller)

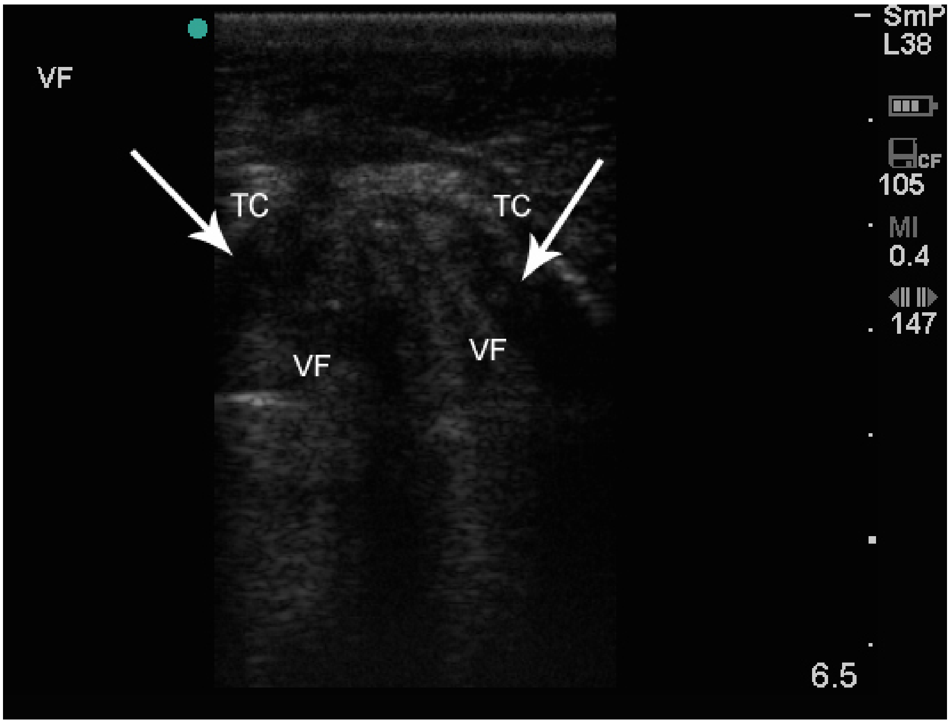

Figure 5-4: Postsurgical 30-50 days ultrasound. Ventricle openings are no longer easily identifiable in this image (white arrows). TC: thyroid cartilage, VF: vocal fold. Right is to the left of the image and skin/ventral is at the top. This is the caudoventral window – transverse plane. This image is from horse LV9. (Image: S. Miller)

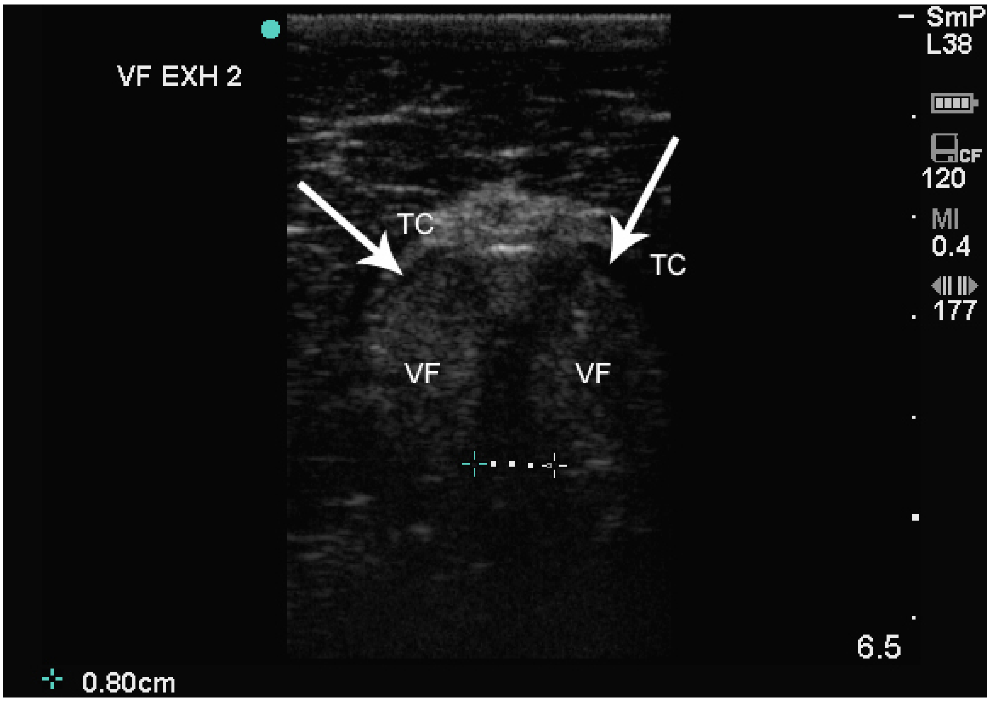

Figure 5-5: Postsurgical 6-12 months ultrasound. Ventricle openings are no longer easily identifiable in this image and appear as thin hypoechoic lines (white arrows). TC: thyroid cartilage, VF: vocal fold. Right is to the left of the image and skin/ventral is at the top. Plusses indicate the measurement of the exhalation vocal fold distance. This is the caudoventral window – transverse plane. This image is from horse LV8. (Image: S. Miller)

The identification of suture material or other prosthetic material proved difficult to evaluate. Of the nine horses that had ethibond prostheses placed, the author found it impossible to definitively identify the suture material in the postsurgical horse. In horse LV8 a different suture material was used which included a metallic crimp tube. This metallic tube was readily visible during ultrasonographic examination in the postsurgical horse (Figure 5-6). The tube is seen as a metallic opacity with a comet tail artefact deep to the tube. The tube measures approximately 1 cm ultrasonographically in the caudolateral window at 3-10 days postsurgery.

Figure 5-6: Left larynx with metallic tube (clip) and comet tail artefact deep to the clip. Plusses measuring the size of the clip at approximately 1 cm in the caudolateral window at 3-10 days postsurgery. Cranial is to the left and the skin surface at top of the image (image: S. Miller)

We can see from the above data that it is possible to detect if a ventriculectomy has been performed. Due to all horses having had a bilateral ventriculectomy it is obviously not possible to analyse the data for difference of detection of laryngoplasty alone versus laryngoplasty with ventriculectomy or ventriculocordectomy.

5.4 HYPOTHESIS 4

Postsurgical complications:

It is possible to detect postsurgical complications of laryngoplasty in the extra-luminal structures, if present.

On ultrasonographic examination, seven horses with recognized complications were detected (Table 5-2). Five of the individual horses that had complications were detected during the examination at 3-10 days postsurgery and they included a seroma (Figure 5-7), haematoma (Figure 5-8) and excessive swelling; these resolved by the following examination. A sixth horse presented with changes to the right VF on ultrasonographic examination at the 30-50 day examination. These changes included hyperechogenicity and thickening of the right VF with luminal ringdown artefacts deep to the thickened area. There was also hyperechognicity of the area surrounding the entrance to the ventricle (Figure 5-9 A). This change was considered to be consistent with abscess formation. On endoscopic examination this was confirmed to be an abscess (Figure 5-9 B). The horse was placed on treatment and the abscess had resolved by the 6-12 month follow up examination both ultrasonographically and endoscopically (Figure 5-10 A&B). A seventh horse presented with changes at the 6-12 month follow up examination. The left VF base showed an increased hyperechoic echotexture, a luminal mass of approximately 0.5 x 0.5 cm with a ringdown artefact deep to the mass, and the artefact projected into the RG area (Figure 5-11 A). This is consistent with a granuloma type mass. Endoscopy confirmed a granuloma type mass at the base of the left VF, superficial to the epiglottis (Figure 5-11 B).

Complications were seen at a rate of 50 % mild complications and 20 % moderate complications. This equates to a total complication rate of 70 %.

Table 5-2: Complications identified ultrasonographically postsurgery

	Excessive swelling (larger than 2cm)	Seroma	Haematoma	Vocal Fold Abscess	Vocal fold granuloma
3-10 days postsurgery	1	3	1	-	-
30-50 days postsurgery	-	-	-	1	-
6-12 months postsurgery	-	-	-	-	1

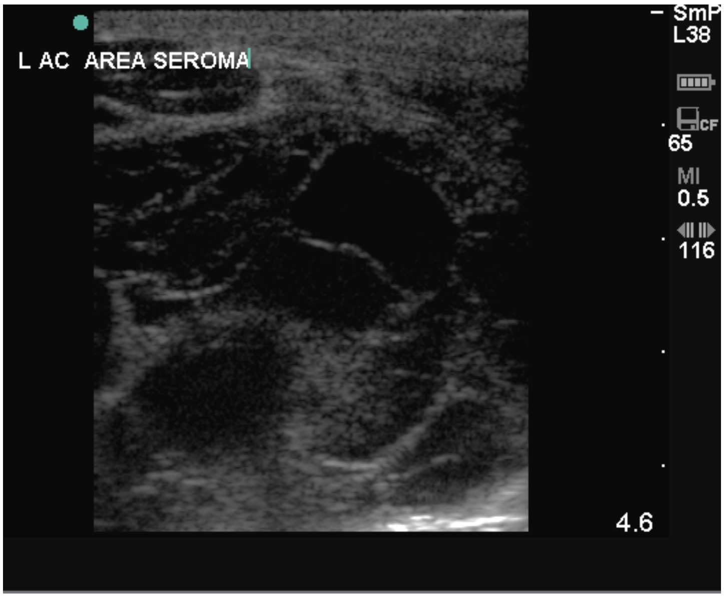

Figure 5-7: Representative image of seroma formation in three out of ten horses. This image is from horse LV8. This is the left caudolateral longitudinal window. The seroma in this area is approximately 4 cm x 4.5 cm in size. Cranial is to the left and the skin surface at top of the image (image: S. Miller)

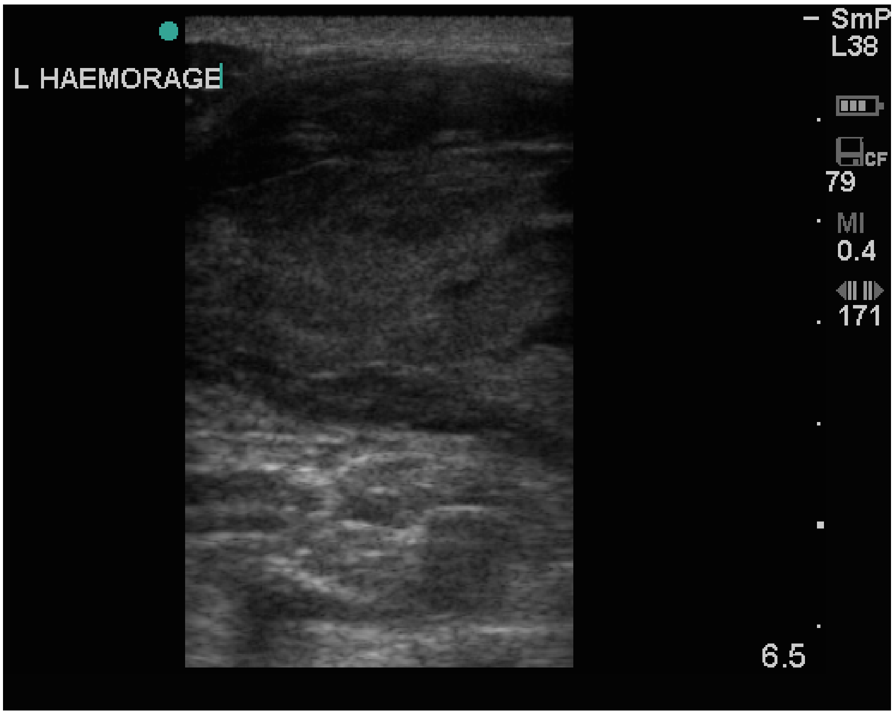

Figure 5-8: Representative image of a haematoma with a heterogeneous echoic appearance, in one out of ten horses. This image is from horse LV7. This is the left caudolateral longitudinal window. Cranial is to the left and the skin surface at top of the image. Note the more echoic 'speckled' pattern differentiating the haemorrhage from seroma formation in Figure 4-5 (image: S. Miller).

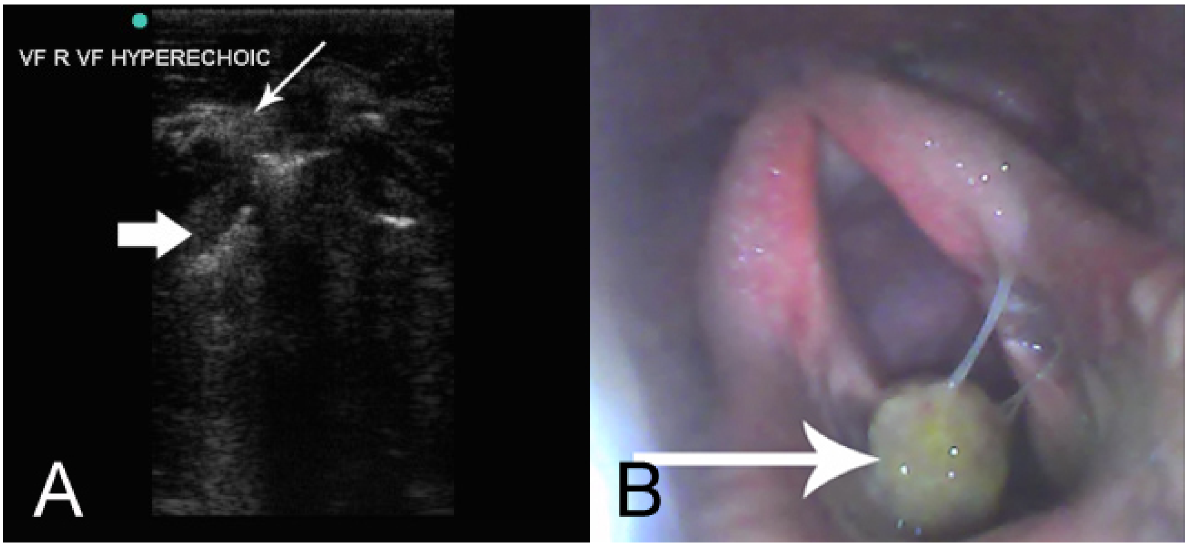

Figure 5-9 **A**: The right vocal fold at 30-50 days postsurgery. The right vocal fold (thick white arrow) is hyperechoic compared to normal, and compared to the left vocal fold in this examination. In addition there are hyperechoic areas surrounding the entrance to the right ventricle (thin white arrow) with luminal ringdown artefacts deep to this mass. Right is to the left of the image and skin/ventral is at the top. This is the caudoventral window – transverse plane. This is image is from horse LV10. (Image: S. Miller). **B**: The corresponding right vocal fold at 30-50 days postsurgery on endoscopic examination. The vocal fold abscess can be clearly seen as a yellowish-greenish mass covered in mucoid material (white arrow). Right is on the left of the image. This is image is from horse LV10. (Image: S. Miller)

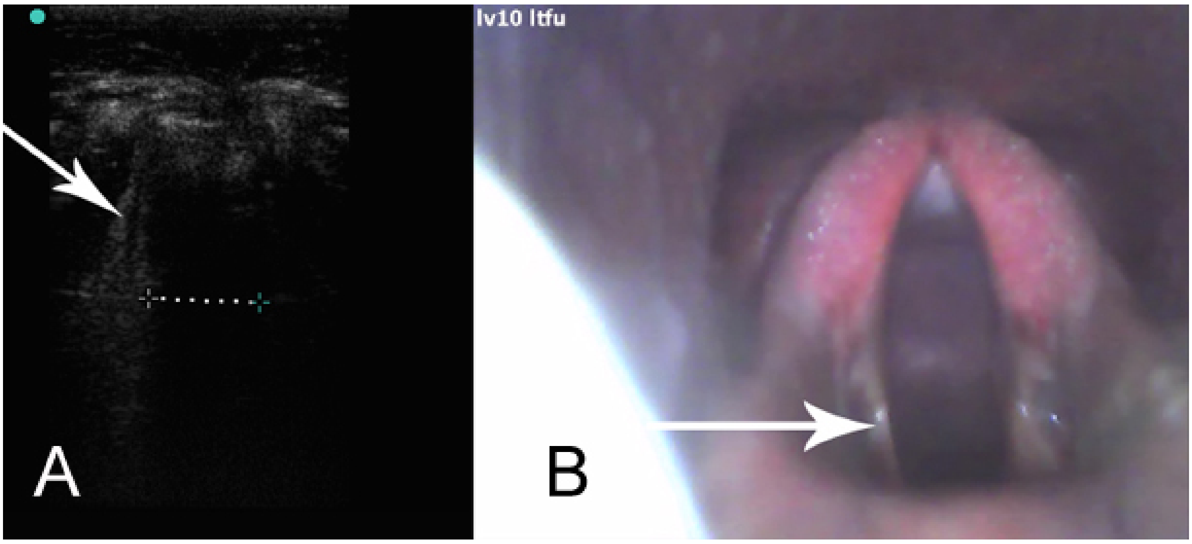

Figure 5-10 **A**: The same vocal folds (from Figure 5-9) examined at 6-12 months. Note the return to normal echogenicity of the vocal fold (white arrow). Right is to the left of the image and skin/ventral is at the top. This is the caudoventral window – transverse plane. Dotted line is measurement of the distance between the vocal folds. This image is from horse LV10. (Image: S. Miller). **B**: The same vocal folds examined at 6-12 months postsurgery on endoscopic examination. There is no longer an abscess on the right vocal fold (white arrow). Right is on the left of the image. This image is from horse LV10. (Image: S. Miller)

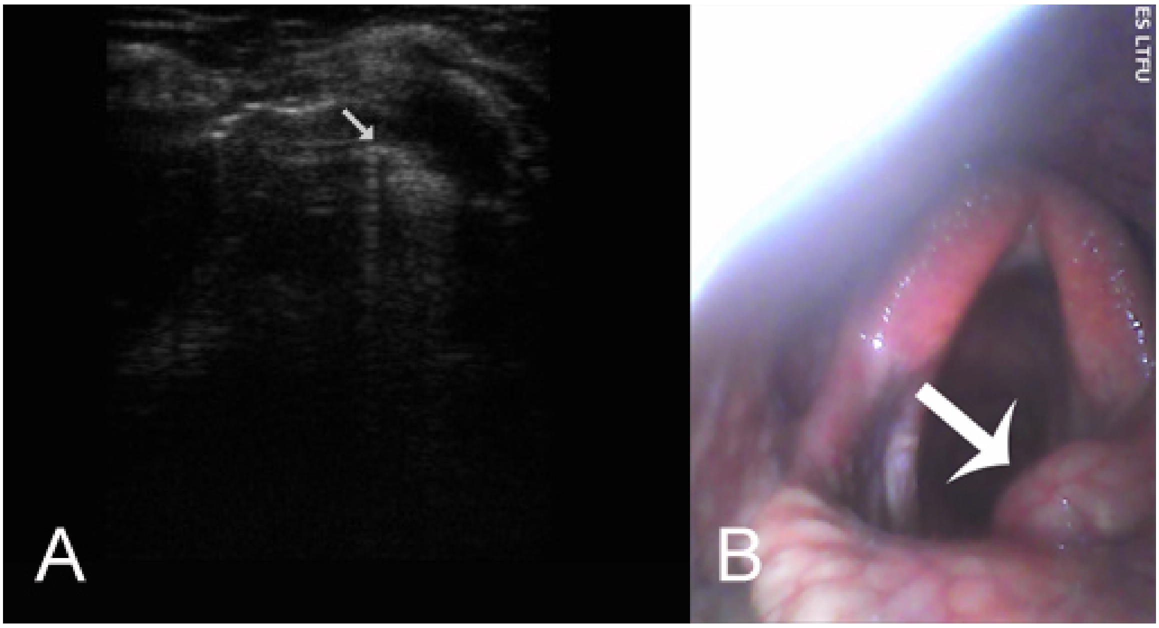

Figure 5-11 **A**: The left vocal fold at 6-12 months postsurgery. This vocal fold base is hyperechoic compared to normal, and compared to the right vocal fold in this examination (white arrow). This is consistent with thickening of the tissue in this area which is attributed to a granuloma. In addition there is a ringdown artefact created by this mass. Right is to the left of the image and the skin/ventral is at the top. This is the caudoventral window – transverse plane. This image is from horse LV3. (Image: S. Miller). **B**: The corresponding right vocal fold at 6-12 months postsurgery on endoscopic examination. The vocal fold granuloma can be clearly seen (white arrow). Right is on the left of the image. This image is from horse LV3. (Image: S. Miller)

The hypotheis was not disproved. The researcher was able to see both extra- and intra-luminal complications.

5.5 HYPOTHESIS 5

Correlation between laryngeal endoscopy examination and laryngeal ultrasonography in the postsurgical larynx:

Upper respiratory endoscopy can be correlated to ultrasonography of the structures visible within the laryngeal lumen

Ultrasonographic VF exhalation distances means and standard deviations were comparing presurgical, 3-10 days and 30-50 days postsurgery (n = 10) and comparing presurgical, 3-10 days, 30-50 days postsurgery and 6-12 months postsurgery (n = 6). There was no statistical difference between the exhalation distances of the three measurement intervals ($\chi^2(2) = 0.889$, $P = 0.641$) in the shorter term data. In the longer term data there was a statistically significant difference in vocal fold exhalation distance ultrasonographically ($\chi^2(3) = 10.8$, $P = 0.013$). A Wilcoxon signed-rank post hoc test showed a statistically significant difference in vocal fold exhalation distance for presurgery compared to 30-50 days postsurgery ($Z = -2.014$, $P = 0.044$) as well as presurgery compared to 6-12 months postsurgery ($Z = -2.201$, $P = 0.028$). The ultrasonographic mean VF exhalation distance increased from 0.67 ± 0.11 cm to 0.96 ± 0.11 cm at the 30-50 day postsurgical examination and from 0.67 ± 0.11 cm to 1.00 ± 0.24 cm at the 6-12 month examination. Vocal fold inhalation distances means and standard deviations were comparing presurgical, 3-10 days and 30-50 days postsurgery (n = 10) and comparing presurgical, 3-10 days, 30-50 days postsurgery and 6-12 months postsurgery (n = 6) . Both showed no significant difference ($\chi^2(2) = 0.462$, $P = 0.794$ and $\chi^2(3) = 3$, $P = 0.392$ respectively). The means and standard deviations are displayed in Table 8-1 (Appendix 8).

Comparison of inhalation versus exhalation mean distance per examination was performed. Presurgical (n = 9) showed a statistically significant difference in VF inhalation vs. exhalation distance ($\chi^2(2) = 10.000$, $P = 0.002$, with the presurgery inhalation mean distance being 0.31 cm larger than presurgery exhalation mean distance. At 3-10 days postsurgery (n = 10) showed a statistically significant difference in VF inhalation vs. exhalation distance ($\chi^2(3) = 10.000$, $P = 0.002$), with the inhalation mean distance being 0.19 cm larger than the exhalation mean distance. At 30-50 days postsurgery showed a statistically significant difference in VF inhalation vs. exhalation distance at 30-50 days ($\chi^2(3) = 5.444$, $P = 0.020$), with the inhalation mean distance being 0.21 cm larger than the exhalation mean distance. 6-12 months (n = 6) showed a statistically significant difference in VF inhalation vs. exhalation distance ($\chi^2(2) = 6.000$, $P = 0.014$), with the inhalation mean distance being 0.25 cm larger than the exhalation mean distance. The means and standard deviations are displayed in Table 9-1 (Appendix 9).

A one-tailed paired Students T-test was performed to determine if the means of the inhalation distance and the exhalation distance were significantly different over time. For 3-10 days and 6-12 months inhalation distance there was a significant difference ($t(5) = -0.235$, $P = 0.026$). For presurgical and 6-12 month exhalation distance there was a significant difference ($t(5) = -0.335$, $P = 0.005$). For 3-10 days and 30-50 days exhalation distance there was a significant difference ($t(5) = -0.223$, $P = 0.034$). For the remaining pairs there was no significant difference. The means and standard deviations for each examination period are displayed in Table 9-2 (Appendix 9). The mean change over time is graphically represented in Figure 5-12.

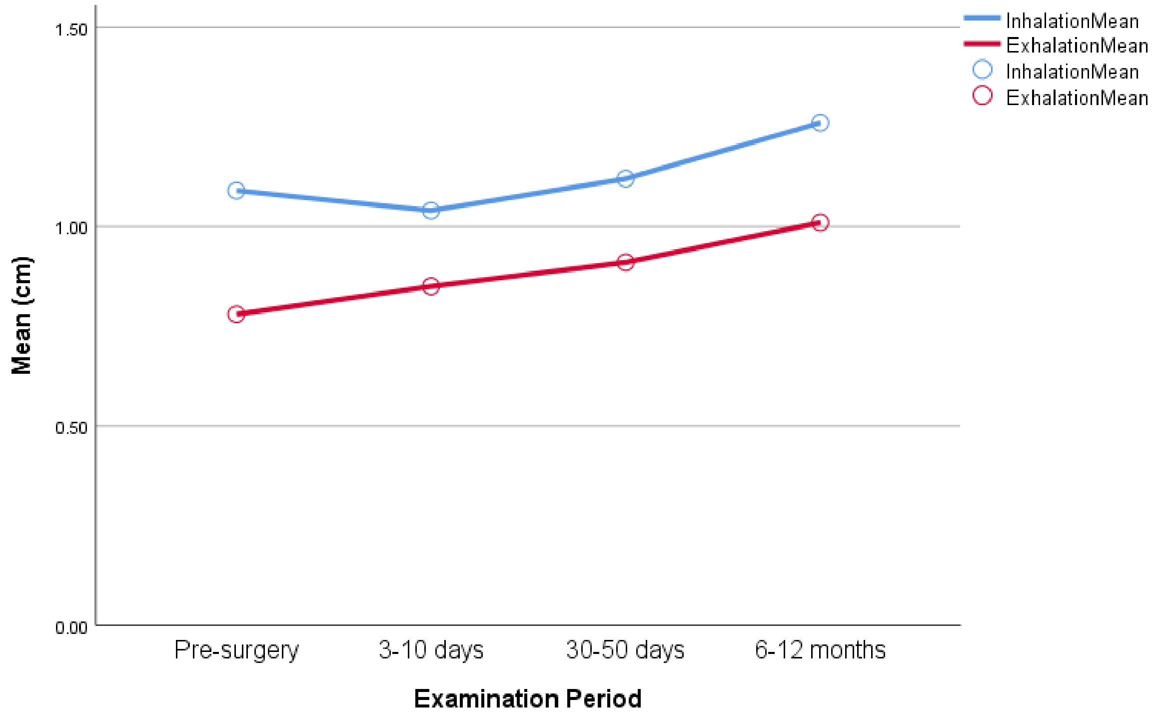

Figure 5-12: Vocal fold inhalation and exhalation distance means (cm) over time for each examination period.

The right VF movement was always graded as present at all examinations for all horses. Presurgical left VF movement was graded as absent for eight out of ten horses, and intermediate for two out of ten horses. For all ten short term examinations left VF movement was absent. For medium term examination left VF movement was graded as intermediate for one out of ten horses and absent for nine out of ten horses. For the long term examination all six horses' left VF movements was graded as absent.

The right AC movement was always graded ultrasonographically as present at all examinations for all horses. Presurgical left AC movement was graded as absent for eight out of ten horses, and intermediate for two out of ten horses. For all ten short term examinations left AC movement was absent. For medium term examination left AC movement was graded as intermediate for one out of ten horses and absent for nine out of ten horses. For the long term examination all six horses' left AC movement was graded as absent.

5.6 HYPOTHESIS 6

Correlation between laryngeal endoscopy examination and laryngeal ultrasonography in the postsurgical larynx:

Extra-luminal structures can be better visualised ultrasonographically than endoscopically

The structures were generally easily evaluated ultrasonographically and swallowing did not affect the ability to evaluate the structures of interest. The only difficulty encountered was when swelling, seroma or haematoma hampered the evaluation of the CAL in some horses and in one horse the AC in the short term postsurgical period.

The transverse BH depth in the medium term data (n = 10) showed a statistically significant difference ($\chi^2(2) = 9.282$, P = 0.010). A Wilcoxon signed-rank *post hoc* test showed a statistically difference in transverse BH depth for 3-10 days compared to 30-50 days postsurgery (Z = -2.075, P = 0.038). The transvers BH depth in the long term data (n = 6) showed a statistically significant difference in transverse BH depth showed a statistically significant difference in transverse BH depth over the long term ($\chi^2(3) = 8.60$, P = 0.035). A Wilcoxon signed-rank *post hoc* test showed a statistically difference in transverse BH depth for presurgery compared with 3-10 days (Z = -2.201, P = 0.028), 3-10 days postsurgery compared with 30-50 days postsurgery (Z = -2.207, P = 0.027) and 3-10 days postsurgery compared with 6-12 months postsurgery (Z = -1.992, P = 0.046). The means and standard deviations for each examination period are displayed in Table 10-1 (Appendix 10).

The caudal BH depth in the medium term (n = 10) did not show a statistically significant difference ($\chi^2(2) = 1.421$, P = 0.491). The caudal BH depth in the long term (n = 6) did not show a significant difference ($\chi^2(3) = 1.33$, P = 0.721.

The caudal BH depth to rostral TC distance in the medium term (n = 10) did not show a statistically significant difference ($\chi^2(2) = 0.359$, P = 0.836). The caudal BH depth to rostral TC distance in the long term (n = 6) did not show a statistically significant difference in ($\chi^2(3) = 0.20$, P = 0.978).

The rostral TC depth in the medium term (n = 10) showed a statistically significant difference ($\chi^2(2) = 6.615$, P = 0.037). A Wilcoxon signed-rank *post hoc* test showed a statistically difference in for 3-10 days compared with 30-50 days (Z = -2.073, P = 0.038). The rostral TC depth in the long term (n = 6) did not show a statistically significant difference in rostral TC depth over the long term ($\chi^2(3) = 4.40$, P = 0.221). The means and standard deviations for each examination period are displayed in Table 10-2 (Appendix 10).

The left CAL circumference, cross-sectional area and widest diameter comparisons did not show a statistically significant difference, for left CAL circumference in the medium term (n = 10) $\chi^2(2) = 0.519$, P = 0.772 and long term (n = 6) $\chi^2(3) = 2.040$, P = 0.564, for left CAL cross-sectional area in the medium term (n = 10) $\chi^2(2) = 2.741$ P = 0.254 and long term (n = 6) $\chi^2(3) = 2.280$, P = 0.516, for left CAL widest diameter in the medium term (n = 10) $\chi^2(2) = 1.543$, P = 0.462 and in the long term (n = 6) $\chi^2(3) = 0.763$, P = 0.858. These were easy to measure ultrasonographically generally, except where inconsistencies associated with postsurgical swelling, seroma or haematoma in some horses made it more difficult to evaluate the CAL. Similarly, the right CAL circumference, cross-sectional area and widest diameter comparisons did not show a statistically significant difference for right CAL circumference in the medium term (n = 10) $\chi^2(2) = 0.667$, P = 0.717 and long term (n = 6) $\chi^2(3) = 1.400$, P = 0.706, for right CAL cross-sectional area in the medium term (n = 10) $\chi^2(2) = 1.897$, P = 0.387 and long term (n = 6) $\chi^2(3) = 2.200$, P = 0.532, for right CAL widest diameter in the medium term (n = 10) $\chi^2(2) = 0.800$, P = 0.670 and in the long term (n = 6) $\chi^2(3) = 1.800$, P = 0.615.

The left AC cross-sectional area was easy to measure ultrasonographically generally, except in the 3-10 days postsurgical period where in some horses it was more difficult to evaluate. The left AC cross-sectional area did not show a statistically significant difference in the medium term (n = 10) $\chi^2(2) = 0.889$, P = 0.641 or the long term (n = 6) $\chi^2(3) = 3.000$, P = 0.392. The right AC cross-sectional area did not show a statistically significant difference in the medium term (n = 10) $\chi^2(2) = 3.800$, P = 0.150 or the long term (n = 6) $\chi^2(3) = 2.600$, P = 0.457.

From the data we can accept our hypothesis that extra-luminal structures can be better visualised ultrasonographically than endoscopically.

Endoscopy only allowed the visualisation of luminal complications. Ultrasonography allowed the visiualisation of both eextra- and intra-luminal complications seen during this study.

In summary, hypothesis 1, 2, 4, 5 and 6 were not disproved. Hypothesis 3 was not possible to evaluate fully.

CHAPTER SIX: DISCUSSION

The data from this study show that post laryngoplasty ultrasonography in the horse was possible and that the normal structures of the horse larynx could be readily identified in the post laryngoplasty horse. Evaluation immediately postsurgery however may be hampered by some common minor postsurgical issues, such as the presence of swelling, seromas and haematomas, which either distort the image or place the structure under investigation too deep to examine with the specific transducer used.

It was anticipated at the outset of the study that the laryngoplasty procedure would have a relatively high complication rate of between 7 % and 43 % [5, 104]. This should have allowed sufficient candidates to form a group consisting of complications. While this study had a fairly high complication rate, the small sample size did not allow for the creation of a separate group.

Only six horses were examined at 6-12 months. For the remainder of the horses it was not logistically possible to follow them longer than 6 months because they were either retired from racing, moved provinces, or sold, making it difficult to trace them.

As expected, the initial postsurgical ultrasonographic examination revealed localized swelling around the surgical site, while some horses had seromas and haematomas fairly early in the course of healing. No gas was noted in the laryngoplasty surgical site. All horses had varying degrees of purulent material lying within the laryngeal lumen, when evaluating the caudoventral window to view the VFs. This was most apparent at the 3-10 days postsurgical examination. The purulent material also drained from the laryngotomy opening. On three ultrasonographic examinations, air appeared to be trapped within the purulent material. This was most likely associated with inhaled or exhaled air which created bubbles within the viscous purulent material. This purulent material was mostly associated with the ventral aspect of the RG but also with the VF edges. The origin of this material appeared to be due to excision of the ventricles, but was also most certainly from the laryngotomy wound. This material had little effect on the examination of the VF and was normal for examination at that stage postsurgery.

Postsurgical examination immediately postsurgery was hampered by the localized swelling resulting in a deeper field depth of the areas to be evaluated as was experienced in a few trial horses before the start of this study. Horses examined closer to three days postsurgery had more swelling than those examined closer to ten days postsurgery. Pain associated with the surgical site in the immediate postsurgical horse also made evaluation more difficult and the use of sedation should then be considered. Most horses tolerated ultrasonographic examination well from three days postsurgery onwards, thus no sedation was needed for examination. Twitching was used to aid endoscopic examination as this had been shown not to affect the grading [14]. The normal structures were much more easily identified the later the postsurgical examination was performed. This was most likely due to the reduction of swelling over time associated with the area.

The data showed that the acoustic windows described in the examination of the normal horse were relevant in examination of the postsurgical laryngoplasty horse. The acoustic windows were therefore validated for their use in the postsurgical evaluation of the equine larynx.

Slight adjustment of placement may need to be made for examining the caudoventral and left caudolateral windows immediately postsurgery. The caudoventral window may be influenced by the length and placement of the ventriculectomy surgical site. Placement of the transducer directly in front of this site allowed easy visualisation of the required structures. The caudolateral windows on the left side may similarly be influenced by the placement of the laryngoplasty surgical site, and potentially the suture material used to close the surgical wound. In the horses in this study, this posed no problems for the examination of the required structures.

The caudolateral window in longitudinal plane proved the most difficult to evaluate during the early postsurgical period. In these examinations the CAL was only imaged in seven out of ten horses. This was most likely due to the increased swelling (horses LV1 and LV10) and haematoma formation (horse LV7) in this area. Delayed evaluation, at 5-10 days, allowed time for initial swelling to subside. With haematoma or seroma formation, a longer period of delay may be required before evaluation is possible. Alternatively, the use of a lower frequency transducer may aid visualisation of deeper structures at an earlier stage.

Similarly, in the caudolateral window transverse plane, the left AC was imaged in nine out of the ten horses. The formation of haematomas in this area (as seen in horse LV7) can hamper optimal visualisation.

Due to the surgical techniques selected by the surgeons for each horse available to study, it was not possible to test if a laryngoplasty alone can be differentiated from a laryngoplasty plus ventriculectomy or ventriculocordectomy using postsurgical ultrasonography. It was also not possible to determine if a normal horse larynx and that of a post laryngoplasty horse larynx can be easily distinguished. It can be difficult to establish if a horse has undergone a laryngoplasty, as a larynx with a prosthesis may have the same appearance as a grade 4 presurgical laryngeal hemiplegia horse with an immobile arytenoid cartilage. Due to the small sample size it was not possible to definitively give a distance for VF inhalation or exhalation excursion that would provide definitive evidence of an increase in the inter VF distance, and in turn the resting RG. In addition, the identification of suture material could be difficult for the inexperienced ultrasonographer to evaluate. Once long term healing had occurred, it was possible to detect if ventricles were present or absent on ultrasonographic examination. This should help determine if a horse has previously undergone a ventriculectomy. The use of metallic clips in the prosthesis would indicate clearly if a prosthesis was in place.

Interestingly, on postsurgical 3-10 days evaluation, horse LV6 and, on 6-12 months evaluation, horse LV10 each had both ventricles still visible. Similarly, both cases of intermediate evaluation of ventricle visibility in both left and right occurred on the same horses (horse LV4 and LV5). While it was not possible to evaluate in this study, it is assumed that the evaluation of ventricles postsurgery could be affected by the skill of the surgeon in removing sufficient ventricular tissue. Incomplete removal will potentially allow the postsurgical ventricle to be indistinguishable from a normal ventricle in the postsurgical examination.

The identification of suture material postsurgery was found to be difficult in this study. Preliminary tests with cadaver specimens also proved identifying the suture material to be difficult. In addition, the selection of material may play a role as composition of the suture material may also influence the ability to visualise it ultrasonographically. The angle of approach to evaluate suture material also has an influence as suture material is more easily seen in the sagittal plane [105] and positioning of the probe in this study may have been inadequate to evaluate the suture material. In nine of the ten horses, a 5 USP synthetic braided coated polyethylene terephthalate (Ethibond[5]) was used as the prosthetic. In the other horse a monofilament nylon with a metallic crimp tube was used (Medium dog cruciate suture with crimp, 80 lb breaking strength[6]). This tube was readily visible on ultrasonographic examination as a metallic echogenicity with a distal comet tail artefact.

Due to the low number of horses available to the researcher, there was limited opportunity to study postsurgical complications. Three of the complications that occurred during the study (seroma, haematoma, excessive swelling) are commonly associated with laryngoplasty and ventriculectomy, and were graded as mild complications. Vocal fold

[5] Ethicon, United States of America
[6] Kyron labs, Johannesburg, South Africa

granuloma formation and VF abscessation were recognised but occurred less commonly and were graded as moderate complications. No severe complications were detected in this study. Due to the low case numbers it was difficult to say for certain if a complication rate of 70 was significant or representative of the surgical procedure in general. In all likelihood, the true complication rate would be far lower. A more realistic approach, based on this study, would be to consider a mild complication rate of 50 % and a moderate complication rate of 20 % separately, as seen in this study. Although these may still not be a true reflection of the postsurgical complication rate, they may be a better reflection of the occurrence of differing severities of complications than a combined total complication rate.

Surgical technique may also play a role in the rate of complications. As one surgeon performed nine of the ten surgeries, comparative evaluation of the technique of the surgeon was not possible in this study. Although it was not possible to perform any statistical analysis, it appeared as if the outcomes of the one horse where the surgery was performed by a different surgeon are similar to the remainder of the group. Higher case numbers and a larger diversity of surgeons performing this procedure would be needed to identify if complications are as a result of a particular surgeon or are associated with the procedure in general.

Abscess formation was distinguishable from granuloma formation due to abscesses typically having an isoechoic or hyperechoic appearance compared to adjacent soft tissue. Identification of an abscess was also aided by the presence of ringdown artefacts in the vicinity, as in case LV10 in this study, or by swirling of internal echoes [106, 107].

Slight adjustment of placement of the probe was needed to allow for optimal visualisation of the caudoventral window at 3-10 days postsurgery. This was done by placing it slightly cranial to the laryngotomy site (through which the ventriculectomy was performed). Exudate from the ventriculectomy, draining out the laryngotomy site may contaminate the probe and the use of a condom or similar protecting structure over the probe can prevent it from becoming soiled. This positioning still allowed clear visualisation of the structures under investigation.

From the data, it was clear that distance between the VFs at both inhalation and exhalation can be assessed. As would be expected when performing a laryngoplasty, there was clear increase in the exhalation distance postsurgery when measured ultrasonographically. The inhalation distance between presurgical and postsurgical VF distance surprisingly showed no statistical difference. This may have been due to the small sample size affecting the ability to detect a significant difference. The data set also contained an outlier which had no influence on the outcome of significance. The distance was measured at rest and not under any deep inhalation. For future studies, it is suggested that a re-breathing bag be used to stimulate deep inhalation to get more accurate measures of the increase in inhalation distance between VFs. In addition, in future studies it is suggested that an endoscopic measurement of RG distance should be measured and compared to the distance measured ultrasonographically.

When looking at each examination, there was a clear difference in the measurements of inhalation versus exhalation mean distance between VFs. In addition, the mean distance increased at each postsurgical examination from 0.19 cm (1.04 cm inhalation distance minus 0.85 cm exhalation distance) at 3-10 days postsurgically, to 0.25 cm (1.26 cm inhalation distance minus 1.01 cm exhalation distance) 6-12 months postsurgically. There was also a significant difference between successive examinations postsurgically. This suggests that a baseline normal mean inhalation and exhalation distance between VFs could be established. In addition, mean distance values could be set that would equate to the endoscopic grading of the success of a laryngoplasty, based on the increased distance achieved between VFs when a laryngoplasty is performed.

Regarding left VF and AC movement, it was interesting to note that both horses that were graded “intermediate” ultrasonographically for both VF and AC movements at the presurgical examination, had endoscopic Havemeyer gradings of 2.2 (horse LV1) and 2.1 (horse LV7). It would appear that at the time of examination there was some movement in the VFs attributed to the movement of the ipsilateral AC. This corresponded to the endoscopic grading. This suggests that an ultrasonographic grading of intermediate for movement would correlate to an endoscopic grading of 2 (and subgrades) using the Havemeyer system. Although not possible to evaluate in this study, this lends some weight to the possibility of producing a reliable ultrasonographic grade for evaluation of the VFs. For medium term examination, VF and AC movement was graded as intermediate for one out of ten horses and absent for nine out of ten horses. This was attributed to difficulty in identifying movement of the VF/AC or absence of VF/AC movement rather than partial movement of these structures.

Evaluation of the extra-laryngeal structures was easily performed ultrasonographically. All structures were easily identifiable and measurable. There were no significant differences in any measurements of a given anatomical structure over time. The only significant differences highlighted by the data were when depth (distance from skin surface to object of interest) was the measurement of interest. This change was most likely due to the swelling resulting from the trauma associated with the surgery and was not a long-standing change once the surgical wound had healed. The significant findings were related to the depth of the BH and the rostral TC depth. Because the remainder of the measurements remained the same, it was clear that a laryngoplasty and ventriculectomy do not affect the non-luminal structures in any significantly measurable way in this study.

There is a clear advantage of ultrasonography over endoscopy in the examination of complications, particularly to the practitioner who does not have access to an endoscope. Ultrasonography is easily able to identify both extra- and intraluminal structures (where the pathology is in contact with the wall of the larynx) and complications, while endoscopy is only able to evaluate intraluminal complications. Endoscopy would allow diagnosis of abscesses or granulomas, as seen in this study; however it is a poor method to evaluate complications like seromas, haematomas or other potential extraluminal complications, although an excessively large haematoma or other extra-luminal mass may exert pressure on the larynx resulting in a narrower laryngeal lumen which could be visualized endoscopically. Ultrasonography would be a superior, less invasive and more easily accessible tool for evaluation of both intra- and extraluminal structures and their complications in the post laryngoplasty horse.

The small population size of this study poses restrictions on the conclusions which can be drawn from the data obtained. In order to detect a significant difference in prevalence of ultrasonographic outcomes, it was not feasible to create more than one group as this would reduce the sample size. The identical surgical procedures performed on all horses also prevented dividing the horses into multiple groups. The sample size limitation was due to the reliance of clinical cases on one main equine clinic with a second clinic providing only one horse. During the planning phase of this study, two other clinics were identified as sources of horses. However due to logistical and other reasons, horses from these clinics were not available for study. There were also changes in the economic climate that had a major impact on the number of clinical cases presented to the primary clinic. This saw a drop in the overall surgical, and specifically laryngoplasty, case load during the study period.

In future, a study with a larger sample size should be conducted. There should be specific focus on comparing the ultrasonographic appearance of ventriculectomy versus ventriculocordectomy. In addition, a larger sample size will allow the comparison of different surgeons' techniques on the postsurgical ultrasonographic appearance of the equine larynx. A larger sample size will also allow for a greater chance to evaluate the detection of complications that were not identified in this study, such as surgical wound infection, failure of the prosthesis to maintain abduction (short- or long-term), prosthetic infection, sinus tract formation, aryepiglottic fold collapse, VF collapse, pharyngeal lymphoid hyperplasia, and upper oesophageal incompetence.

The establishment of a grading system for ultrasonographic evaluation of the success of the laryngoplasty procedure would aid clinicians in the field, particularly those that do not have easy access to an endoscope.

A standardised measuring system for ultrasonographic evaluation of the distance between VFs should be established. This requires a larger sample size to determine the normal distance as well as the distance in a laryngeal hemiplegia horse and a horse postsurgery for both inhalation and exhalation values. Baseline mean distance between VF values could be assigned that would correlate to the endoscopic grading of the success of a laryngoplasty, based on the increased distance achieved between vocal folds when a laryngoplasty is performed.

CHAPTER SEVEN: CONCLUSION

This study concludes that:

i. post laryngoplasty ultrasonographic examination of the equine larynges is possible and that the structures described in a normal laryngeal ultrasonographic examination can be easily seen;
ii. the ultrasonographic windows described by previous authors for the normal are valid for use in the post laryngoplasty horse;
iii. it is possible to determine if a ventriculectomy has been performed on an equine when examined on postsurgical ultrasonography;
iv. it is possible to detect postsurgical complications of laryngoplasty in the extra-luminal structure ultrasonographically, when present;
v. it is possible to detect postsurgical complications of laryngoplasty in the intra-luminal structure ultrasonographically, when present;
vi. it is possible to examine the vocal folds, ventricles and the majority of the arytenoid cartilage by means of ultrasonography. While there are clear links between the appearance of the vocal folds endoscopically and ultrasonographically more research into this area could result in an ultrasonographic grading system for distance between vocal folds, which could be standardised to be used as an ultrasonographic grading system for the assessment of success of a laryngoplasty; and
vii. the extra-luminal structures are easily identifiable ultrasonographically in the postsurgical horse. This allows easy monitoring of these structures for complications that may arise related to the surgery.

REFERENCES

1. Morris, E. and Seeherman, H. (1991) Clinical evaluation of poor performance in the racehorse: the results of 275 evaluations. *Equine Vet. J.* **23,** 169-74.

2. Kannegieter, N. and Dore, M. (1995) Endoscopy of the upper respiratory tract during treadmill exercise: a clinical study of 100 horses. *Aust. Vet. J.* **72,** 101-107.

3. Martin, B.J., Reef, V., Parente, E. and Sage, A. (2000) Causes of poor performance of horses during training, racing, or showing: 348 cases (1992-1996). *J. Am. Vet. Med. Assoc.* **216,** 554-558.

4. Tan, R., Dowling, B. and Dart, A. (2005) High-speed treadmill videoendoscopic examination of the upper respiratory tract in the horse: the result of 291 clinical cases. *Vet. J.* **170,** 243-248.

5. Dixon, P., McGorum, B., Railton, D., Hawe, C., Tremaine, W., Dacre, K. and McCann, J. (2003) Long-term survey of laryngoplasty and ventriculocordectomy in an older, mixed-breed population of 200 horses. Part 1: Maintenance of surgical arytenoid abduction and complications of surgery. *Equine Vet. J.* **35,** 389-396.

6. Hawkins, J., Tulleners, E., Ross, M., Evans, L. and Raker, C. (1997) Laryngoplasty with or without ventriculectomy for treatment of left laryngeal hemiplegia in 230 racehorses. *Vet. Surg.* **26,** 484-491.

7. Boyle, A., Martin, B., Davidson, E., Durando, M. and Birks, E. (2006) Dynamic pharyngeal collapse in racehorses. *Equine Vet. J.* **38,** 546-550.

8. Barakzai, S. (2016) Equine laryngeal dysplasia. *Equine Vet. Educ.* **28,** 276-283.

9. Franklin, S., Naylor, J. and Lane, J. (2006) Videoendoscopic evaluation of the upper respiratory tract in 93 sport horses during exercise testing on a high-speed treadmill. *Equine Vet. J.* **38,** 540-545.

10. Kelly, P., Reardon, R., Johnston, M. and Pollock, P. (2013) Comparison of dynamic and resting endoscopy of the upper portion of the respiratory tract in 57 Thoroughbred yearlings. *Equine Vet. J.* **45,** 700-704.

11. Allen, K. and Franklin, S. (2010) Comparisons of overground endoscopy and treadmill endoscopy in UK Thoroughbred racehorses. *Equine Vet. J.* **42,** 186-191.

12. Barakzai, S. and Cheetham, J. (2012) Endoscopic examination of exercising horses: effects on diagnosis and treatment of upper respiratory tract disorders. *Equine Vet. J.* **44,** 501-503.

13. Ducharme, N., Hackett, R., Fubini, S. and Erb, H. (1991) The reliability of endoscopic examination in assessment of arytenoid cartilage movement in horses. Part II. Influence of side of examination, reexamination, and sedation. *Vet. Surg.* **20,** 180-184.

14. Archer, R., Lindsay, W. and Duncan, I. (1991) A comparison of techniques to enhance the evaluation of equine laryngeal function. *Equine Vet. J.* **23,** 104-107.

15. Lindegaard, C., Husted, L., Ullum, H. and Fjeldborg, J. (2007) Sedation with detomidine and acepromazine influences the endoscopic evaluation of laryngeal function in horses. *Equine Vet. J.* **39,** 553-556.

16. McCarrel, T. and Woodie, J. (2015) Update on laryngeal disorders and treatment. *Vet. Clin. North Am. : Equine Prac.* **31,** 13-26.

17. Garrett, K. (2012) Advances in diagnostic imaging of the larynx and pharynx. *Equine Vet. Educ.* **24,** 17-18.

18. Linford, R., Meagher, D.M., O'Brien, T. and Wheat, J. (1983) Radiographic assessment of epiglottic length and pharyngeal and laryngeal diameters in the Thoroughbred. *Am. J. Vet. Res.* **44,** 1660-1666.

19. Smith, C. (2009) Diagnostic techniques used to evaluate the upper respiratory tract in horses. *Aust. Equine Vet.* **28,** 54-55.

20. Tatarniuk, D., Carmalt, J. and Allen, A. (2010) Induration of the cricoid cartilage complicates prosthetic laryngoplasty in a horse. *Vet. Surg.* **39,** 128-130.

21. Pekarkova, M., Kircher, P., Konar, M., Lang, J. and Tessier, C. (2009) Magnetic resonance imaging anatomy of the normal equine larynx and pharynx. *Vet. Radiol. Ultrasound.* **50,** 392-397.

22. Dahlberg, J., Valdes â Martinez, A., Boston, R. and Parente, E. (2011) Analysis of conformational variations of the cricoid cartilages in Thoroughbred horses using computed tomography. *Equine Vet. J.* **43,** 229-234.

23. Tulloch, L.K., Piercy, R.J., Troester, S., Carruthers, R., Tast, V., Grimes, L. and Perkins, J.D. (2014) Use of laryngeal computed tomography for noninvasive assessment of laryngeal function in horses with recurrent laryngeal neuropathy. *Equine Vet. J.* **S47,** 17.

24. Garrett, K., Woodie, J., Embertson, R. and Pease, A. (2009) Diagnosis of laryngeal dysplasia in five horses using magnetic resonance imaging and ultrasonography. *Equine Vet. J.* **41,** 766-771.

25. Holmes, S. (2014) Equine skull magnetic resonance imaging: The where, when and why? *Equine Vet. Educ.* **26,** 605-609.

26. Tucker, R. and Sampson, S. (2007) Magnetic resonance imaging protocols for the horse. *Clin. Tech. Equine. Pract.* **6,** 2-15.

27. Chalmers, H., Cheetham, J., Yeager, A. and Ducharme, N. (2006) Ultrasonography of the equine larynx. *Vet. Radiol. Ultrasound.* **47,** 476-481.

28. Garrett, K. (2010) How to ultrasound the equine larynx. *American Association of Equine Practitioners Proceedings.* **56,** 249-256.

29. Chalmers, H., Yeager, A., Cheetham, J. and Ducharme, N. (2012) Diagnostic sensitivity of subjective and quantitative laryngeal ultrasonography for recurrent laryngeal neuropathy in horses. *Vet. Radiol. Ultrasound.* **53,** 660-666.

30. Chalmers-Chaudhry, H. (2014) The use of ultrasonography for assessment of the equine intrinsic muscles. *Doctor of Philosophy dissertation.* **University of Guelph,** Ontario, Canada.

31. Bienert-Zeit, A., Roetting, A., Reichert, C. and Ohnesorge, B. (2014) Laryngeal fistula formation after laryngoplasty in two Warmblood mares. *Equine Vet. Educ.* **26,** 88-92.

32. Chalmers, H., Yeager, A. and Ducharme, N. (2009) Ultrasonographic assessment of laryngohyoid position as a predictor of dorsal displacement of the soft palate in horses. *Vet. Radiol. Ultrasound.* **50,** 91-96.

33. Garrett, K., Embertson, R., Woodie, J. and Cheetham, J. (2013) Ultrasound features of arytenoid chondritis in Thoroughbred horses. *Equine Vet. J.* **45,** 598-603.

34. Solano, M. and Penninck, D. (1996) Ultrasonography of the canine, feline and equine tongue: normal findings and case history reports. *Vet. Radiol. Ultrasound.* **37,** 206-213.

35. Wong, K., Lang, B., Ng, S., Cheung, C., Chan, C. and Lo, C. (2013) A prospective, assessor-blind evaluation of surgeon-performed transcutaneous laryngeal ultrasonography in vocal cord examination before and after thyroidectomy. *Surgery.* **154,** 1158-1164.

36. Barakzai, S., Dixon, P., Hawkes, C., Cox, A. and Barnett, T. (2015) Upper esophageal incompetence in five horses after prosthetic laryngoplasty. *Vet. Surg.* **44,** 150-155.

37. Brown, J., Dereksen, F., Stick, J., Hartmann, W. and Robinson, N. (2004) Effect of laryngoplasty on respiratory noise reduction in horses with laryngeal hemiplegia. *Equine Vet. J.* **36,** 420-425.

38. Cramp, P., Derksen, F.J., Stick, J.A., Nickels, F.A., Brown, K.E., Robinson, P. and Robinson, N.E. (2009) Effect of ventriculectomy versus ventriculocordectomy on upper airway noise in draught horses with recurrent laryngeal neuropathy. *Equine Vet. J.* **41,** 729-734.

39. Dixon, P., McGorum, B., Railton, D., Hawe, C., Tremaine, W., Pickles, K. and McCann, J. (2002) Clinical and endoscopic evidence of progression in 152 cases of equine recurrent laryngeal neuropathy (RLN). *Equine Vet. J.* **34,** 29-34.

40. Kidd, J. and Slone, D. (2002) Treatment of laryngeal hemiplegia in horses by prosthetic laryngoplasty, ventriculectomy and vocal cordectomy. *Vet. Rec.* **150,** 481-484.

41. Cramp, P. and Barakzai, S. (2012) Surgical management of recurrent laryngeal neuropathy. *Equine Vet. Educ.* **24,** 307-321.

42. Brown, J., Derkson, F., Stick, J., Hartmann, W. and Robinson, N. (2003) Ventriculocordectomy reduces respiratory noise in horses with laryngeal hemiplegia. *Equine Vet. J.* **35,** 570-574.

43. Carpenter, R., McIlwraith, C. and Hill, A. (2009) Racing performance in 72 racehorses treated with prosthetic laryngoplasty for laryngeal hemiplegia. *J. Equine Vet. Sci.* **29,** 584-589.

44. Barakzai, S. (2015) When is ventriculocordectomy all that is required? *Equine Vet. Educ.* **27,** 218-219.

45. Taylor, S., Barakzai, S. and Dixon, P. (2006) Ventriculocordectomy as the sole treatment for recurrent laryngeal neuropathy: long-term results from ninety-two horses. *Vet. Surg.* **35,** 653-657.

46. Barnett, T., O'Leary, J., Parkin, T., Dixon, P. and Barakzai, S. (2013) Long-term exercising video-endoscopic examination of the upper airway following laryngoplasty surgery: a prospective cross-sectional study of 41 horses. *Equine Vet. J.* **45,** 593-597.

47. Davidson, E., Martin, B., Boston, R. and Parente, E. (2011) Exercising upper respiratory videoendoscopic evaluation of 100 nonracing performance horses with abnormal respiratory noise and/or poor performance. *Equine Vet. J.* **43,** 3-8.

48. Compostella, F., Tremaine, W. and Franklin, S. (2012) Retrospective study investigating causes of abnormal respiratory noise in horses following prosthetic laryngoplasty. *Equine Vet. J.* **44,** 27-30.

49. Kraus, B., Parente, E. and Tulleners, E. (2003) Laryngoplasty with ventriculectomy or ventriculocordectomy in 104 draft horses (1992-2000). *Vet. Surg.* **32,** 530-538.

50. Robinson, P., Dereksen, F., Stick, J., Sullins, K., Detolve, P. and Robinson, N. (2006) Effects of unilateral laser-assisted ventriculocordectomy in horses with laryngeal hemiplegia. *Equine Vet. J.* **38,** 491-496.

51. Brown, J., Derksen, F., Stick, J., Hartmann, W. and Robinson, N. (2005) Laser vocal cordectomy fails to effectively reduce respiratory noise in horses with laryngeal hemiplegia. *Vet. Surg.* **34,** 247-252.

52. Henderson, C., Sullins, K. and Brown, J. (2007) Transendoscopic, laser-assisted ventriculocordectomy for treatment of left laryngeal hemiplegia in horses: 22 cases (1999-2005). *J. Am. Vet. Med. Assoc.* **231,** 1868-1872.

53. Strand, E., Martin, G., Haynes, P., McClure, J. and Vice, J. (2000) Career racing performance in Thoroughbreds treated with prosthetic laryngoplasty for laryngeal neuropathy: 52 cases (1981-1989). *J. Am. Vet. Med. Assoc.* **217,** 1689-1696.

54. Barakzai, S., Boden, L. and Dixon, P. (2009) Race performance after laryngoplasty and ventriculocordectomy in National Hunt racehorses. *Vet. Surg.* **38,** 941-945.

55. Bathe, A. (1993) Left laryngeal hemiplegia in the horse: a survey of diagnostic criteria and management practices employed by 20 veterinary surgeons in Europe. *Equine Vet. Educ.* **5,** 84-85.

56. Sweeney, C. (1992) Left laryngeal hemiplegia in the horse: a survey of diagnostic criteria and management practices employed by 25 veterinarians in the United States. *Equine Vet. Educ.* **4,** 93-95.

57. Ahern, B. and Parente, E. (2008) Surgical complications of the equine upper respiratory tract. *Vet. Clin. North Am. : Equine Prac.* **24,** 465-484.

58. Davenport-Goodall, C. and Parente, E. (2003) Disorders of the larynx. *Vet. Clin. North Am. : Equine Prac.* **19,** 169-187.

59. Froydenlund, T. and Dixon, P. (2014) A review of equine laryngoplasty complications. *Equine Vet. Educ.* **26,** 98-106.

60. Hardcastle, M., Pauwels, F. and Collett, M. (2012) Clinicopathologic observations on laryngoplasty failure in a horse. *Vet. Surg.* **41,** 649-653.

61. Davenport, C., Tulleners, E. and Parente, E. (2001) The effect of recurrent laryngeal neurectomy in conjunction with laryngoplasty and unilateral ventriculocordectomy in Thoroughbred racehorses. *Vet. Surg.* **30,** 417-421.

62. Barnett, T., O'Leary, J., Parkin, T., Dixon, P. and Barakzai, S. (2013) Long-term maintenance of arytenoid cartilage abduction and stability during exercise after laryngoplasty in 33 horses. *Vet. Surg.* **42,** 291-295.

63. Mason, B., Riggs, C. and Cogger, N. (2013) Cohort study examining long-term respiratory health, career duration and racing performance in racehorses that undergo left-sided prosthetic laryngoplasty and ventriculocordectomy surgery for treatment of left-sided laryngeal hemiplegia. *Equine Vet. J.* **45,** 229-234.

64. Garrett, K., Woodie, J. and Embertson, R. (2011) Association of treadmill upper airway endoscopic evaluation with results of ultrasonography and resting upper airway endoscopic evaluation. *Equine Vet. J.* **43,** 365-371.

65. Maurits, N., Bollen, A., Windhausen, A., De Jager, A. and Van der Hoeven, J. (2003) Muscle ultrasound analysis: Normal values and differentiation between myopathies and neuropathies. *Ultrasound Med. Biol.* **29,** 215-225.

66. Chalmers, H., Caswell, J., Perkins, J., Goodwin, D., Viel, L., Ducharme, N. and Piercy, R. (2016) Ultrasonography detects early laryngeal muscle atrophy in an equine neurectomy model. *Muscle Nerve.* **53,** 583-592.

67. Vavasseur, P., Lechartier, A., Robert, C. and Mespoulhes, C. (2017) Development of a quantitative method for ultrasonographic assessment of recurrent laryngeal neuropathy in horses. *Bull Acad Vet Fr.* **170,** 118-125.

68. Robinson, N. (2004) Consensus statements on equine recurrent laryngeal neuropathy: conclusions of the Havemeyer workshop. *Equine Vet. Educ.* **16,** 333-336.

69. Collins, N., Milne, E., Hahn, C. and Dixon, P. (2009) Correlation of the Havemeyer endoscopic laryngeal grading system with histopathological changes in the equine cricoarytenoideus lateralis muscle. *Irish Vet. J.* **62,** 334-338.

70. Jiménez-Díaz, F., Jimena, I., Luque, E., Mendizábal, S., Bouffard, A., Jiménez-Reina, L. and Peña, J. (2012) Experimental muscle injury: correlation between ultrasound and histological findings. *Muscle Nerve.* **45,** 705-712.

71. Karlheim, B., Barton, A., Rohn, K. and Ohnesorge, B. (2015) Validity of laryngeal ultrasonography in reference to endoscopy at rest and during exercise in Warmblood horses. *Equine Vet. Educ.* **27,** 86-91.

72. O'Neill, H., Ballegeer, E., De Feijter-Rupp, H., Stick, J., Derksen, F. and Robinson, N. (2014) Ultrasound-guided biopsy of the cricoarytenoideus lateralis muscle: technique and safety in horses. *Equine Vet. J.* **46,** 244-248.

73. Pillen, S., Verrips, A., van Alfen, N., Arts, I., Sie, L. and Zwarts, M. (2007) Quantitative skeletal muscle ultrasound: Diagnostic value in childhood neuromuscular disease. *Neuromuscul. Disord.* **17,** 509-516.

74. Pillen, S., Arts, I. and Zwarts, M. (2008) Muscle ultrasound in neuromuscular disorders. *Muscle Nerve.* **37,** 679-693.

75. Simon, N., Ralph, J., Lomen-Hoerth, C., Poncelet, A., Vucic, S., Kiernan, M. and Kliot, M. (2015) Quantitative ultrasound of denervated hand muscles. *Muscle Nerve.* **52,** 221-230.

76. Kelly, P. (2016) Studies on serial resting and dynamic endoscopic examination of Thoroughbred yearlings. *Master of Veterinary Medicine dissertation.* **University of Glasgow,** Glasgow.

77. Chalmers, H., Viel, L., Caswell, J. and Ducharme, N. (2015) Ultrasonographic detection of early atrophy of the intrinsic laryngeal muscles of horses. *Am. J. Vet. Res.* **76,** 426-436.

78. Kraft, M. and Arens, C. (2008) Technique of high-frequency endolaryngeal ultrasound. *J. Laryngol. Otol.* **122,** 1109-1111.

79. Fjordbakk, C., Chalmers, H., Holcombe, S. and Strand, E. (2013) Results of upper airway radiography and ultrasonography predict dynamic laryngeal collapse in affected horses. *Equine Vet. J.* **45,** 705-710.

80. Ahern, B., Boston, R. and Parente, E. (2012) *In vitro* mechanical testing of an alternate laryngoplasty system (ALPS) for horses. *Vet. Surg.* **41,** 918-923.

81. Lechartier, A., Rossignol, F., Brandenberger, O., Vitte, A., Mespoulhès-Rivière, C., Rossignol, A. and Boening, K. (2015) Mechanical comparison of 3 anchoring techniques in the muscular process for laryngoplasty in the equine larynx. *Vet. Surg.* **44,** 333-340.

82. Raffetto, J., Wearn, J. and Fischer, A. (2015) Racing performance following prosthetic laryngoplasty using a polyurethane prosthesis combined with a laser-assisted ventriculocordectomy for treatment of recurrent laryngeal neuropathy in 78 Thoroughbred racehorses. *Equine Vet. J.* **47,** 60-64.

83. Biasutti, S., Dart, A. and Jeffcott, L. (2017) A review of recent developments in the clinical application of prosthetic laryngoplasty for recurrent laryngeal neuropathy: Indications, complications and outcome. *Equine Vet. Educ.* **29,** 337-345.

84. Gomaa, M., Kramer, M., Samy, M., Omar, M. and Hekkawy, N. (2012) Ultrasonographic findings of most common surgical disorders of gastrointestinal tract in dogs and cats. *Iranian J. Vet. Surg.* **7,** 23-38.

85. Matthews, A.R., Penninck, D.G. and Webster, C.R.L. (2008) Postoperative ultrasonographic appearance of uncomplicated enterotomy or enterectomy sites in dogs. *Vet. Radiol. Ultrasound.* **49,** 477-483.

86. Norbury, J., Warren, K. and Moore, D. (2014) Tarsal tunnel syndrome secondary to suture material within the tibial artery. *Am. J. P. M. R.* **93,** 361.

87. Prickett, W., Teefey, S., Galatz, L., Calfee, R., Middleton, W. and Yamaguchi, K. (2003) Accuracy of ultrasound imaging of the rotator cuff in shoulders that are painful postoperatively. *J. Bone Jt. Surg.* **85-A,** 1084-1089.

88. Wilson, D.A., Badertscher, R.R., Boero, M.J., Baker, G.J. and Foreman, J.H. (1989) Ultrasonographic evaluation of the healing of ventral midline abdominal incisions in the horse. *Equine Vet. J.* **s7,** 107-110.

89. Jann, H., Blaik, M., Emerson, R., Tomioka, M., Stein, L. and Moll, D. (2003) Healing characteristics of deep digital flexor tenorrhaphy within the digital sheath of horses. *Vet. Surg.* **32,** 421-430.

90. Hulley, S.B., Cummings, S.R., Bronwer, W.S., Grady, D.G. and Newman, T.B. (2007) Designing clinical research 3rd Edition. **Lippincott Williams and Wilkins, Philadelphia,** 65-96.

91. Dixon, P.M., Robinson, N. and Wade, J.F. (2004) Proceedings of a workshop on equine recurrent laryngeal neuropathy. **R and W Publishers,** Suffolk, UK.

92. Rakestraw, P., Hackett, R., Ducharme, N., Nielan, G. and Erb, H. (1991) Arytenoid cartilage movement in resting and exercising horses. *Vet. Surg.* **20,** 122-127.

93. Davidson EJ, Martin BB, Rieger RH and Parente EJ. (2010) Exercising videoendoscopic evaluation of 45 horses with respiratory noise and/or poor performance after laryngoplasty. *Vet. Surg.* **39,** 942-948.

94. Ghasemi, A. and Zahediasl, S. (2012) Normality tests for statistical analysis: a guide for non-statisticians. *Int. J. Endocrinol. Metabol.* **10,** 486-489.

95. Pillai, K.C.S. (1955) Some new test criteria in multivariate analysis. *Ann. Math. Stat.* **26,** 117-121.

96. Mauchly, J.W. (1940) Significance test for sphericity of a normal n-variate distribution. *Ann. Math. Stat.* **11,** 204-209.

97. Greenhouse, S.W. and Geisser, S. (1959) On methods in the analysis of profile data . *Psychometrika.* **24,** 95-112.

98. Shaffer, J.P. (1995) Multiple hypothesis testing. *Annu. Rev. Psychol.* **46,** 561-584.

99. Wittkowski, K.M. (1988) Friedman-type statistics and consistent multiple comparisons for unbalanced designs with missing data. *J. Am. Stat. Assoc.* **83,** 1163-1117.

100. Wilcoxon, F. (1945) Individual comparisons by ranking methods. *Biometrics Bulletin.* **1,** 80-83.

101. Cochran, W.G. (1950) The comparison of percentages in matched samples. *Biometrika.* **37,** 256-266.

102. Koo, T.K. and Li, M.Y. (2016) A guideline of selecting and reporting intraclass correlation coefficients for reliability research. *J Chiropr Med.* **15,** 155-163.

103. Student. (1908) The probable error of a mean. *Biometrika.* **6,** 1-25.

104. Dixon, P. (2004) Evaluation of RLN treatment efficiency - Laryngoplasty. **R and W Publishers,** Stratford-upon Avon, UK.

105. Parulekar, S.G. and Kiwi, R. (1982) Ultrasound evaluation of sutures following cervical cerclage for incompetent cervix uteri. *J. Ultrasound Med.* **1,** 223-228.

106. Loyer, E.M., Kaur, H., David, C.L., DuBrow, R.A. and Eftekhari, F. (1995) Importance of dynamic assessment of the soft tissues in the sonographic diagnosis of echogenic superficial abscesses. *J. Ultrasound Med.* **14,** 669-671.

107. Loyer, E.M., DuBrow, R.A., David, C.L., Coan, J.D. and Eftekhari, F. (1996) Imaging of superficial soft-tissue infections: sonographic findings in cases of cellulitis and abscess . *Am J Roentgenol.* **166,** 149-152.

APPENDIX 1 PARTICIPATING VETERINARIAN PERMISSION

Permission of Principal Partner or Participating Veterinarian to allow the veterinary clinic and clinical cases to be used in this MSc research study: Ultrasound examination of the post laryngoplasty horse

Dear Doctor,

Thank you for giving me permission to use your cases and your practice to perform ultrasound on these cases.

The study aims to investigate and improve the ultrasonographic knowledge of the equine larynx. The normal and diagnosis of certain abnormalities have been well described. This study will evaluate the post laryngoplasty appearance of the larynx, appearance of any possible complications and aim to be able to identify a successful laryngoplasty in the long-term.

Please read through the requirements below and sign this form in agreement that you are willing to participate in this study using your cases.

I, ______________________ (full name) of ______________________ (clinic) understand that ultrasonography will be performed at the following stages of the procedure and agree to allow Dr Miller access to, or help to gain access to, the horse at these times to perform the required procedures:

Day prior to/day of surgery presurgery
Day 3-10 postsurgery
Day 30-50 postsurgery
As far as possible 3-4 months, 6-7 months and 12-14 months postsurgery

The ultrasonography will be performed free of charge.
The surgeon or the referring veterinarian is requested to perform 3 endoscopic video recordings of the larynx: Presurgical video, immediate postsurgical video (3-10 days postsurgery), long-term postsurgical video 30-50 days postsurgery). Please supply Dr Miller with the 3 videos for each case presented. Grading of the videos will be done by Dr Miller. If any of the horse present with any postsurgical complications related to the surgical procedure (i.e. implant failure, infection or

draining tract/fistula formation) please notify Dr Miller as soon as possible so that this horse can be ultrasounded.

Your help will be acknowledged in the dissertation and in any publications arising from this research. The results of the research will also be made available to you. Thank you for the opportunity to ultrasound these horses.

…………………………………… ……………………………………

Signature Date

PLEASE EMAIL COMPLETED FORM TO SEAN@SAVETS.CO.ZA

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA
Faculty of Veterinary Science

Permission of owner / agent to have horse included in the study: Ultrasound examination of the post laryngoplasty horse

The above research project is being performed to increase our knowledge of the ultrasonographic appearance of the horse larynx post tie-back.

I, ………………………………………………….., the owner / agent of the …………….. year-old Thoroughbred, …..……………….. (gender) horse, named ………………………………… on this ………………… (date) at…………………..….(place) give permission that the horse have ultrasound and endoscopy performed of its larynx, as part of an MSc project evaluating the larynx of horses, admitted to …………………Veterinary Clinic respectively. Any sedation required for this procedure will be given according to standard practices used in these clinics, and will be administered free of charge.

I understand that ultrasound examinations will be performed at the following stages of the procedure and agree to allow Dr Miller access to the horse at these times to perform the required procedures:

Day prior to/day of surgery
Day 3-10 postsurgery
Day 30-50 postsurgery
As far as possible 3-4 months, 6-7 months and 12-14 months postsurgery

The ultrasound will be performed free of charge while the horse is being evaluated for whatever other reason it was referred to the relevant clinic. If a report of the findings is required, it can be requested from Dr S Miller (primary researcher). No report will be supplied otherwise. The advantage of being part of this study is that you will be able to assess the healing of your horse as it recovers from its tie-back procedure and contribute to knowledge regarding this procedure.

……………………………………… ………………………………………

Signature Date

PLEASE EMAIL COMPLETED FORM TO SEAN@SAVETS.CO.ZA

Physical examination

Horse Details				
Date:		**Experimental Number:**		
Horse name:			Age:	
Trainer:		Owner:		
Clinical Evaluation				
Attitude of horse:				
Is there evidence of laboured breathing? if yes please describe:				
Pulse rate:		Pulse rhythm:		
Pulse Character:		Resp. rate:		
Mucous Membrane colour:			CRT	
Symmetrical airflow from nostrils:			yes	no
Auscultation of larynx:				
Auscultation of trachea:				
Palpation of larynx:				

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APPENDIX 4 PRESURGICAL ENDOSCOPIC GRADING

Hospital admittance and presurgical endoscopic grading

Horse Details				
Date:		**Experimental Number:**		
Horse name:			Age:	
Trainer:		Owner:		
Clinical reasoning for admission:				
Date of diagnosis of laryngeal hemiplegia:				
Name of Veterinarian to diagnose laryngeal hemiplegia:				
Static endoscopy performed:			Yes	No
Dynamic exercise endoscopy performed:			yes	no
Static Havemeyer grading (please circle appropriate grade) (Robinson 2004):				
Grade 1	All arytenoid cartilage movements are synchronous and symmetrical and full arytenoid cartilage abduction can be achieved and maintained.			
Grade 2	Arytenoid cartilage movements are asynchronous and/or larynx is asymmetric at times but full arytenoid cartilage abduction can be achieved and maintained.			
Grade 2.1	Transient asynchrony, flutter or delayed movements are seen			
Grade 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.			
Grade 3	Arytenoid cartilage movements are asynchronous and/or asymmetric. Full arytenoid cartilage abduction cannot be achieved and maintained			
Grade 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.			

This document forms part of the MSc Post Laryngoplasty ultrasonography of the horse larynx. If found please return to Dr Sean Miller, sean@savets.co.za 0828826593

Grade 3.2	Obvious arytenoid abductor deficit and arytenoid asymmetry. Full abduction is never achieved.			
Grade 3.3	Marked but not total arytenoid abductor deficit and asymmetry with little arytenoid movement. Full abduction is never achieved.			
Grade 4	Complete immobility of the arytenoid cartilage and vocal fold.			
Dynamic Havemeyer Exercise grading (circle appropriate grade) (Robinson 2004):				
Grade A	Full abduction of the arytenoid cartilages during inspiration			
Grade B	Partial abduction of the affected arytenoid cartilages (between full abduction and the resting position).			
Grade C	Abduction less than resting position including collapse into the contralateral half of the rima glottidis during inspiration.			
Surgeon:		Ventriculectomy	Cordectomy	Laryngoplasty
Comments:				

APPENDIX 5

PRESURGICAL ULTRASOUND GRADING

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Faculty of Veterinary Science

Presurgical ultrasound examination

Date of examination:		**Experimental Number:**	
Anatomical location	**Repeated measurement number**		
Transverse Basihyoid bone depth @ Lingual Process junction	1:	2:	3:
Rostrocaudal	1:	2:	3:
Caudal BH bone depth			
Rostral thyroid cartilage depth			
Distance btwn BH & TC			
Left vocal fold, arytenoid, cricoarytenoideus lateralis (CAL) and cricoarytenoideus dorsalis (CAD) assessment (Lateral Acoustic window) in longitudinal plane			
Left Vocal fold Movement:	present	absent	intermediate
Inhalation distance between vocal folds	1:	2:	3:
Exhalation distance between vocal folds	1:	2:	3:
Left CAL widest diameter:	1:	2:	3:
Left CAL circumference:	1:	2:	3:
Left CAL cross sectional area:	1:	2:	3:
Left Arytenoid abduction	present	absent	intermediate
Left Arytenoid cartilage cross sectional area:	1:	2:	3:

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Left AC margin Description:			
Right vocal fold, arytenoid, cricoarytenoideus lateralis (CAL) and cricoarytenoideus dorsalis (CAD) assessment (Lateral Acoustic window) in longitudinal plane			
Right Vocal fold Movement:	present	absent	intermediate
Right CAL widest diameter:	1:	2:	3:
Right CAL circumference:	1:	2:	3:
Right CAL cross sectional area:	1:	2:	3:
Right Arytenoid abduction	present	absent	intermediate
Right Arytenoid cartilage cross sectional area:	1:	2:	3:
Right AC margin Description:			
Ventricle opening visible:	Left	Right	

APPENDIX 6
POSTSURGICAL ULTRASOUND GRADING

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA
Faculty of Veterinary Science

Postsurgical ultrasound examination

Date of examination:		**Experimental Number:**		
Indicate examination stage	3-10 days	30-50 days		6-12 months
Anatomical location		Repeated measurement number		
Transverse Basihyoid bone depth @ Lingual Process junction		1:	2:	3:
Rostrocaudal		1:	2:	3:
Caudal BH bone depth				
Rostral thyroid cartilage depth				
Distance btwn BH & TC				
Left vocal fold, arytenoid, cricoarytenoideus lateralis (CAL) and cricoarytenoideus dorsalis (CAD) assessment (Lateral Acoustic window) in longitudinal plane				
Left Vocal fold Movement:		present	absent	intermediate
Inhalation distance between vocal folds		1:	2:	3:
Exhalation distance between vocal folds		1:	2:	3:
Left CAL widest diameter:		1:	2:	3:
Left CAL circumference:		1:	2:	3:
Left CAL cross sectional area:		1:	2:	3:
Left Arytenoid abduction		present	absent	intermediate

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Left Arytenoid cartilage cross sectional area:	1:	2:	3:
Left AC margin Description:			
Right vocal fold, arytenoid, cricoarytenoideus lateralis (CAL) and cricoarytenoideus dorsalis (CAD) assessment (Lateral Acoustic window) in longitudinal plane			
Right Vocal fold Movement:	present	absent	intermediate
Inhalation distance between vocal folds	1:	2:	3:
Exhalation distance between vocal folds	1:	2:	3:
Right CAL widest diameter:	1:	2:	3:
Right CAL circumference:	1:	2:	3:
Right CAL cross sectional area:	1:	2:	3:
Right Arytenoid abduction	present	absent	intermediate
Right Arytenoid cartilage cross sectional area:	1:	2:	3:
Right AC margin Description:			
Ventricle opening visible:	Left	Right	
Prosthesis visible: yes/no	complications:		
Prosthesis apparent length:	1:	2:	3:

APPENDIX 7

POSTSURGICAL ENDOSCOPY

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA
Faculty of Veterinary Science

Endoscopic appearance postsurgery

Horse Details			
Date:		**Experimental Number:**	
Indicate examination stage	3-10 days	30-50 days	6-12 months
Horse name:		Age:	
Name of Veterinarian to diagnose performing postsurgical endoscopy:			
Postsurgical correction grading (Hawkins *et al.* 1997; Rakestraw *et al.* 1991; Davidson *et al.* 2010)			
Grade 1	Close to or at maximal abduction contacting and depressing the pharyngeal wall (80°-90° to the sagittal plane)		
Grade 2	High degree of arytenoid abduction, contacting the pharyngeal wall (50°-80° to the sagittal plane)		
Grade 3	Moderate abduction, not touching the pharyngeal wall (45° to the sagittal plane)		
Grade 4	Slight degree of arytenoid abduction (slightly more than a resting position)		
Grade 4	No abduction		
Any complications relating to surgery:			
Comments:			

APPENDIX 8 VOCAL FOLD INHALATION MEANS AND STANDARD DEVIATIONS

Table 8-1: Vocal fold inhalation means and standard deviations

	Mean (cm)	**Standard deviation**
Vocal fold exhalation distance (n = 10)		
Presurgery	0.82	0.28
3-10 days postsurgery	0.85	0.17
30-50 days postsurgery	0.91	0.12
Vocal fold exhalation distance (n = 6)		
Presurgery	0.67 * ‡	0.11
3-10 days postsurgery	0.78	0.14
30-50 days postsurgery	0.96 *	0.11
6-12 months postsurgery	1.00 ‡	0.24
Vocal fold inhalation distance (n = 10)		
Presurgery	1.09	0.32
3-10 days postsurgery	1.04	0.15
30-50 days postsurgery	1.11	0.18
Vocal fold inhalation distance (n = 6)		
Presurgery	1.01	0.28
3-10 days postsurgery	1.02	0.16
30-50 days postsurgery	1.17	0.22
6-12 months postsurgery	1.26	0.20

*, ‡ Significantly different ($P < 0.05$)

APPENDIX 9 VOCAL FOLD INHALATION AND EXHALATION DISTANCE AND MEAN AND STANDARD DEVIATION FOR INHALATION AND EXHALATION

Table 9-1: Vocal fold inhalation and exhalation distance per examination

	Mean (cm)	Standard deviation
Presurgery inhalation	1.09 *	0.32
Presurgery exhalation	0.78 *	0.28
3-10d inhalation	1.04 †	0.15
3-10d exhalation	0.85 †	0.16
30-50d inhalation	1.12 ‡	0.19
30-50d exhalation	0.91 ‡	0.12
6-12 m Inhalation	1.26 ±	0.20
6-12 m Exhalation	1.01 ±	0.24

*, †, ‡, ± Significantly different ($P < 0.05$)

Table 9-2: Mean and standard deviation for inhalation and exhalation at each examination point

	Mean Inhalation distance (cm)	Mean exhalation distance (cm)
Presurgery	1.09 ± 0.32 ǂ	0.78 ± 0.28
3-10 days postsurgery	1.04 ± 0.15 * †	0.85 ± 0.16
30-50 days postsurgery	1.12 ± 0.19 †	0.91 ± 0.12
6-12 months postsurgery	1.26 ± 0.2 * ǂ	1.01 ± 0.24

*, †, ǂ Significantly different ($P < 0.05$)

APPENDIX 10 TRANSVERSE BASIHYOID BONE AND ROSTRAL THYROID CARTILAGE DEPTH MEANS AND STANDARD DEVIATION

Table 10-1: Transverse basihyoid bone depth means and standard deviation

	Mean (cm)	Standard deviation
Medium Term (n = 10)		
Presurgery	1.61	0.22
3-10 days postsurgery	1.85 ±	0.28
30-50 days postsurgery	1.64 ±	0.14
Long Term (n = 6)		
Presurgery	1.62*	0.24
3-10 days postsurgery	1.93* ‡ †	0.32
30-50 days postsurgery	1.66 ‡	0.13
6-12 months postsurgery	1.60 †	0.14

±, *, ‡, † Significantly different (P < 0.05)

Table 10-2: Rostral thyroid cartilage depth means and standard deviations

	Mean (cm)	Standard deviation
Medium Term (n = 10)		
Presurgery	4.30	0.72
3-10 days postsurgery	3.67 *	0.53
30-50 days postsurgery	3.24 *	0.48
Long Term (n = 6)		
Presurgery	4.30	0.90
3-10 days postsurgery	3.12	0.48
30-50 days postsurgery	3.24	0.55
6-12 months postsurgery	2.65	0.36

* Significantly different (P < 0.05)