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Computed tomographic assessment of canine arytenoid lateralization

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COMPUTED TOMOGRAPHIC ASSESSMENT OF CANINE ARYTENOID
LATERALIZATION

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LIST OF ABBREVIATIONS

ATW Arytenoid to thyroid wing distance

CAL cricoarytenoid lateralization

CauAM Caudal arytenoid displacement

cCSA Rostral to central cross sectional area of the larynx

CLA Conserved laryngeal axis

CT Computed Tomography

CTAL Cricothyroarytenoid lateralization

CTJ Cricothyroid joint

EMG Electromyographic

IA band Interarytenoid sesamoid band

IAD Interarytenoid band distance

iLP Idiopathic laryngeal paralysis

fid horiz Horizontal displacement of the arytenoid fiducial marker

fid vert Vertical displacement of the arytenoid fiducial marker

MIP Maximal intensity projection

MNCV Motor nerve conduction velocities

RG *Rima glottidis*

RGA Area of the *Rima glottidis*

TAL Thyroarytenoid lateralization

UAL Unilateral arytenoid lateralization

ABSTRACT

Unilateral arytenoid lateralization is a commonly performed surgical treatment for laryngeal paralysis in dogs. It involves fixing the moveable arytenoid cartilage to the thyroid (TAL) or cricoid (CAL) cartilage or both (CTAL). This increases the area of the *rima glottidis* (RGA), to allow reduced airway pressure and laryngeal resistance *in vitro* and ameliorates clinical signs *in vivo*. It may also increase the patient's predisposition for aspiration pneumonia, which occurs in around 20% of clinical patients. No surgical technique has been correlated with clinical outcome or risk of aspiration pneumonia. Objective analysis of the effects of surgery on the three dimensional structure of the larynx has not been performed. Non-invasive assessment and standardization or classification of arytenoid lateralization techniques would allow more effective prospective clinical trials to identify prognostic factors for outcome and complications. Eight cadaver larynges were secured to radiolucent materials for Computed Tomography (CT) before and after TAL, CAL and CTAL with sutures tensioned to 100g or 500g.

Multiple measurements were taken from CT 3D reconstructions of the larynx to assess arytenoid displacement in three separate planes. No significant changes were found for any CT measure except the distance between the arytenoid and thyroid wing (ATW). CTAL at 500g and TAL at 500g showed significantly smaller ATW compared to CAL at 100g suggesting that a high tension TAL or CTAL causes the most lateralization of the arytenoid. CAL may allow reduction in airway pressure without excessive

lateralization of the arytenoid. ATW is a candidate for a marker of lateralization of UAL procedures, which could be implemented in future prospective clinical studies. Sequential tensioning and loosening of the suture had no significant effect on any measured parameter validating the use of larynges in sequential measurements.

1. INTRODUCTION

Laryngeal paralysis is most commonly an idiopathic[1] disease of older large breed dogs that causes inspiratory dyspnea, hyperthermia, exercise intolerance, inspiratory stridor and dysphonia. For the majority of dogs, idiopathic laryngeal paralysis (iLP) is regarded as part of a generalized polyneuropathy[2] complex which also affects swallowing function and may make dogs predisposed to aspiration of food and fluid. Conservative management may allow a sedentary lifestyle in some dogs however surgery is recommended for any dog with an owner perceived reduction in quality of life. Unilateral arytenoid lateralization (UAL), where the movable arytenoid cartilage is sutured at tension to the more caudal cricoid or more lateral thyroid cartilage[3] is the most commonly performed surgery for laryngeal paralysis. Patients have an approximately 20% risk of developing aspiration pneumonia[4] within the perioperative period. It is postulated that insufficient lateralization of the arytenoid cartilage will result in continued dyspnea while excessive lateralization will cause further predisposition to aspiration pneumonia by mismatch of the epiglottis and glottis, which normally creates a tight seal to protect against the leakage of oropharyngeal contents[5]. No information exists regarding the effects of arytenoid lateralization on the 3 dimensional motion of the arytenoid cartilage and the volume of the larynx. Previous *in vitro* and *in vivo* studies have characterized the effect of surgery on *rima glottidis* area (RGA), which the area for air passage between the vocal folds and arytenoid cartilages [6,7-9], airway resistance[5] and airway pressure[10]. No study has been able to correlate surgical technique or objectively measured parameters with outcome. A non-invasive

measure could provide a prospective marker for investigations into the true effect of technique on outcome.

Computed tomography (CT) can be performed with minor sedation in awake animals and allows the delineation between the cartilages of the larynx[11] and has been utilized to diagnose multiple causes of upper airway obstruction including iLP[12].

The aim of this study is to characterize the effect of arytenoid lateralization techniques and suture tension on 3 dimensional motion of the arytenoid cartilage, laryngeal volume and to see if CT could be a useful tool for assessing arytenoid lateralization surgeries for future prospective evaluation in the small number of cases typically seen in veterinary studies.

2. REVIEW OF LITERATURE

2.1. Laryngeal paralysis

The larynx has a number of functions, the most important of which are the optimization of airflow during inspiration, vocalization, and prevention of aspiration of food or liquids during swallowing[13]. iLP is a common disease primarily affecting older large breed dogs and involves the loss of the normal arytenoid cartilage abduction during inspiration due to paralysis of the recurrent laryngeal and nerve and loss of the function of the *cricoartytenoid dorsalis* muscle, amongst others[13]. Congenital or inherited forms have been reported in the Bouvier de Flandres [14], German Shepherd[15], Rottweiler[16] and Siberian husky [17]. Additionally, other breeds have been implicated in association with polyneuropathies. The majority of cases are suggested to be acquired[13]. Causes of acquired laryngeal paralysis include trauma, neoplasia, iatrogenic causes such as radiotherapy and most commonly idiopathic[1](iLP).

2.2. The relevance of airway mechanics and inspiration to laryngeal function

The recurrent laryngeal nerve and *cricoarytenoid dorsalis* muscle are actively responsible for opening the *rima glottidis* (RG) on inspiration, allowing negative pressure generated during expansion of the thoracic cavity during inspiration to move air along a pressure gradient into the respiratory tract. The laryngeal musculature is also responsible for closing the RG when the dog swallows, allowing a secure fit between the glottis and epiglottis and preventing aspiration of flood or fluid. This loss of

dynamic function is both crucial in the pathological processes involved in the disease process, as well as on the diagnosis on laryngeal examination under a light plane of anesthesia[1].

Inspiration is initiated by chest expansion and the negative pressure within the chest creates a differential pressure across the walls of the airway, which includes the trachea and the larynx[18]. The trachea has some structural protection because of its c-shaped rings, the overlap of laryngeal cartilages and the attachment of various ligamentous structures, which may resist collapse. The flow of air exists in two forms- laminar flow and turbulent flow. Laminar flow, where air travels in a single direction, exists with a proportional relationship between flow and pressure[18]. Turbulent flow, where multi-directional flow predominates, has a different relationship and pressure is proportional to the square of flow in the airway. The switch between laminar and turbulent flow is indicated by a Reynolds number of over 2000, which takes into account a number of variables (the radius and diameter of a tube, viscosity of gas and flow rate). Importantly it is generally thought that only air in terminal bronchioles exists in laminar flow[18]. The radius of the tube also has a huge effect on resistance to airflow shown by the following equation

$R = 8nl/pr^4$, where R is resistance, n is gas viscosity, l is length of the passage, and r is radius of the tube. It is clear that in animals with respiratory disease, which suffer from airway narrowing, a situation of increased airflow rates and airway pressure exists and dyspnea can easily become a terminal event. The pressure gradient across the walls of

the airway may itself cause narrowing of the airway, with a halving of airway radius causing a 16-fold increase in inspiratory pressure. Dynamic laryngeal collapse has been reported in exercising horses[19]. In addition, application of enough negative pressure to a cadaver larynx and proximal tracheal construct can cause laryngeal collapse which appears to be centered at the RG[10]. Whether this is occurring in dyspneic canines or whether this only occurs in excised canine larynges at airflow rates and airway pressure above physiological levels is unknown. In the clinical situation, visualization of the larynx itself generally only occurs during a laryngeal exam at a light plane of anesthesia, by which point clinical dyspnea has abated.

During laminar flow along a tube, the flow profile is parabolic, i.e. fastest at the center and slowest at the periphery[18]. This is the case for a cylinder but for both the larynx and trachea, this is an oversimplification[20]. Dekker observed that physical models fabricated from laryngeal–tracheal casts experienced laminar-to-turbulent flow transitions at significantly lower flow rates than their straight tube counterparts[21]. Another study of the internal surface of computerized models of the human trachea found that the presence of cartilage rings in the trachea has a significant effect on airflow and particle deposition[20]. Martonen[22] identified a distinct laryngeal jet, i.e. the narrowing of the RG, has a substantial effect of airflow to the lower airways[23]. In a human 3D larynx model used to investigate phonation, high pressure gradients lead to increased airway velocity and whether the glottis is parallel, divergent or convergent has an effect on the pressure difference [24]. This may suggest that changes in the internal surface and volume of the canine larynx may be a source of airway resistance in addition to the RG.

2.3. Denervation of the larynx and polyneuropathy

The traditional etiopathologic theory relating to laryngeal paralysis were based on equine hemiplegia, the equivalent disease in horses, where a 'distal back' axonopathy[25] was proposed because the long axons in the recurrent laryngeal nerve would be relatively susceptible. However, in canines, examination of previous and current literature implicates a more important systemic disease rather than one restricted to a single pair of nerves or muscles.

Despite the common misconception that the only affected muscle and nerve in laryngeal paralysis are the *cricoarytenoideus dorsalis* and recurrent laryngeal nerve respectively, an experimental investigation from 1933[26] showed that experimental denervation of the recurrent laryngeal nerve resulted in instant constriction of the glottis and severe dyspnea, rather than the dynamic inspiratory obstruction which occurs due to loss of muscular tone in the larynx as seen in iLP patients. The reason for this is due to the balance of muscular tone in the larynx; the RGA is affected dynamically by the muscular balance between the abductor of the larynx (*cricoarytenoid dorsalis*) supplied by the recurrent laryngeal nerves and the adductors of the larynx (all other intrinsic laryngeal muscles, particularly the *cricothyroideus*), which are supplied by the para-recurrent nerves. The para-recurrent laryngeal nerves also supply the cervical and cranial thoracic esophagus and have a role in the intricately coordinated process of swallowing. With the loss of abductor tone in an otherwise normal larynx leads to tight constriction of the adductors and narrowing of the airway, which is not characteristic of iLP. In these experiments, further sectioning of the para-recurrent laryngeal nerves, i.e.

inducing multiple nerve dysfunction, resulted in the arytenoids maintaining a paralyzed median position as seen in iLP.

Due to the emergency presentation of a subset of iLP patients, a full neurologic exam is often not performed or not deemed interpretable due to hypoxia and weakness. One study looked at patients presenting with laryngeal paralysis and sought to investigate whether there were any documentable signs of a concurrent polyneuropathy[2]. Age matched controls were compared to dogs with laryngeal paralysis. This study examined electromyographic (EMG) examination of the left thoracic and left pelvic limbs, pharyngeal and esophageal musculature as well as motor nerve conduction velocities (MNCV) of the left tibial and right ulnar nerves. All dogs in the iLP group had neurological deficits and or electromyographic changes associated with polyneuropathy, which were more consistent with axonal degeneration[2]. A study by Thieman revealed histopathologic changes in both the muscles and nerves of dogs with only respiratory signs[27]. Pathologic changes were present in muscle biopsies from all dogs evaluated. Muscle fiber atrophy, large nerve fiber loss, axonal degeneration, and endoneurial fibrosis confirmed the diagnosis of polyneuropathy due to chronic axonal degeneration and nerve fiber loss in this group of dogs with laryngeal paralysis.

2.4 Esophageal disease and aspiration pneumonia in laryngeal paralysis patients

Dogs with iLP often present to the hospital with a concurrent history of dysphagia and coughing after drinking and the most common complication of surgery for laryngeal paralysis is aspiration pneumonia[4,28,29]. A study by Stanley et al. specifically investigated

esophageal disease in iLP patients compared to aged matched controls[30]. The results showed that based on blinded but subjective grading of an esophagram, abnormalities in the liquid phase of swallowing showed that dysphagia was located in the cranial esophagus. A significant difference was found in esophagram scores for dogs with iLP compared to controls and these findings were more predictive of aspirating after surgery than a full neurological examination. Two control dogs with relatively high esophagram scores went on to develop iLP within 6 months. This study adds weight to the idea of concurrent para-recurrent nerve degeneration, given the function of those nerves in swallowing.

An important part of the swallowing process is the closure and coverage of the RG when swallowing to prevent aspiration of fluid and food into the respiratory system. One *in vitro* study hypothesized that deformation of the RG due to unilateral arytenoid lateralization techniques could lead to a mismatch between the RG and the epiglottis[31], leading to an 'aperture' through which food or fluid may be more likely to be aspirated. The same group was then able to demonstrate a difference in closed glottal resistance for the same procedures- i.e. how well did the seal between the RG and epiglottis resist airflow[27]. These studies both investigated the effect of suture tension for cricoartytenoid lateralization (CAL) and found that increased tension on the suture opened the 'aperture' significantly and reduced closed glottal resistance, both of which were hypothesized to predispose to aspiration pneumonia, however this has not been demonstrated in clinical studies.

On further examination of the etiology of aspiration pneumonia, multiple reasons have been postulated.

- Pre-existing esophageal disease related to generalized polyneuropathy and concurrent disruption of the para-recurrent laryngeal nerves[27,28,30,32].

- Upper respiratory edema both from high pressure during dyspnea as well as iatrogenic damage during surgery and anesthesia.

- Increased inspiratory pressure seen in dyspneic animals has been linked with increased gastro-esophageal reflux.

- As unilateral arytenoid lateralization results in deformation of the shape of the RG and holds it in a fixed position, the creation of a less airtight seal has been postulated to allow aspiration[5].

- Although it is known that a large amount of these cases present with esophageal dysfunction, it is unknown whether the surgical approach itself results in further damage to the nerves or muscles associated with the cranial oesophagus and may increase the severity of dysphagia in some patients.

These recent findings suggest a particularly important cause for post-operative morbidity and mortality and a crucial new challenge for the laryngeal surgeon; how we can improve airflow through the larynx without detrimentally increasing the risks of aspiration pneumonia.

2.5 Surgical treatments for idiopathic Laryngeal Paralysis

Numerous surgical techniques exist for treatment including castellated laryngofissure [33], nerve-muscle pedicle grafts [34], partial laryngectomy [35], bilateral TAL with vocal fold excision and mucosoplasty [36], bilateral ventriculocordectomy via ventral laryngotomy[37] and unilateral and bilateral arytenoid lateralization [29,38]. Nitinol stenting[39] has also been evaluated in a cadaver model.

- Castellated laryngofissure [33]

This utilizes a ventral approach to increase the volume of the larynx and therefore abduct the arytenoid and enlarge the RG. This surgery has largely been abandoned due to the high risk of severe laryngeal edema and bleeding, and the necessity for a perioperative tracheostomy. It is the only previous surgery to utilize an increase in laryngeal volume indirectly to increase the RGA.

- Nerve-muscle pedicle grafting[34]

This technique was used on a model of experimental transection one of the recurrent laryngeal nerves to cause unilateral laryngeal paralysis. The authors used a graft of the 1st cervical nerve and *sternothyroideus* muscle onto the denervated muscle after 1 week. An observer blindly measured arytenoid abduction and found that although better than controls at 19 weeks, abduction was still significantly worse than before surgery. This has not been used clinically due to the slow onset of its effects but has been used in humans with laryngeal muscle palsy[40].

- Partial laryngectomy [35]

  An oral approach can be utilized to allow vocal cord resection with partial arytenoidectomy. Rates of aspiration pneumonia were significantly higher than with arytenoid lateralization in one study[35].

- Bilateral TAL with vocal fold excision and mucosoplasty [36]

  This technique was able to reduce the risk of aspiration pneumonia but major postoperative complications were surgical failures; 12/67 surgeries failed with mean recurrence of clinical signs was at 19 weeks (range 2–30 weeks). Minor complications occurred in 22 (33%) of dogs and proved less successful than arytenoid lateralization with regard to the requirement for repeated surgeries.

- Bilateral ventriculocordectomy via ventral laryngotomy[37]

  A recent study documented a ventral approach to the larynx, allowing symmetrical ventriculocordectomy; the authors subjectively evaluated this as a technically less demanding procedure than arytenoid lateralization and with a lower rate of clinical aspiration pneumonia compared to historical controls. No other controls were present

  and no monitoring was performed for subclinical aspiration pneumonia, although this remains to be compared prospectively to other surgical techniques in clinical patients.

- Unilateral Arytenoid Lateralization[4,5,7-9,29,31,41,42]

Unilateral arytenoid lateralization (UAL) involves a lateral (or less commonly ventral followed by lateral) approach to the larynx to allow one arytenoid cartilage (usually the left arytenoid cartilage) to be abducted out of the airway replacing the function of the *cricoarytenoid dorsalis* muscle using suture material. The arytenoid cartilage is permanently sutured to a more external cartilage of the larynx. This has emerged as a preferred technique due to ease of procedure and good reported clinical outcome [29,42].

- Nitinol stenting[39]

Small self-expanding nitinol stents were placed into the arytenoid cartilages of an excised cadaver larynx. A significant reduction in airway resistance was maintained but the approach to apply such a stent would be difficult; so far no documented clinical trials have been performed.

2.6 Clinical outcome and complication rates for laryngeal surgery

The largest retrospective study of iLP patients evaluated the outcome of 140 cases and looked at multiple different surgeries. Overall these patients suffered a 34.3% complication rate and 14.3% dogs died of disease-related causes. Aspiration pneumonia occurred in 23.6% and seven dogs died of aspiration pneumonia more than 1 year after surgery. Complication and mortality rate were increased by bilateral arytenoid lateralization surgeries, patient age, temporary tracheostomy placement, concurrent respiratory tract abnormalities, concurrent esophageal disease, postoperative

megaesophagus, concurrent neoplastic disease, and concurrent neurologic disease. [28] A study of 40 dogs which were subject to UAL by a single experienced surgeon showed that 18% of cases suffered post-operative aspiration pneumonia; in this study 6/7 affected by aspiration pneumonia recovered. Minor complications were observed in 56% of dogs and included unresolved coughing or gagging, continued exercise intolerance, vomiting and seroma formation were the most common. In this study, 90% of owners reported an improvement in postoperative quality-of-life score. Median survival time was 12 months; only 1 dog was euthanized because of respiratory tract disease following surgery.[4]

A similar study from another surgeon took retrospective data from sixty-two dogs over a three-year period. The perioperative complication rate was approximately 10%, while the success rate as judged by owners one year postoperatively was greater than 90 per cent.[29]

2.7 Measurement techniques for evaluation of Unilateral Arytenoid Lateralization

Owner or veterinarian assessment of outcomes of a surgical procedure is useful but subjective and potentially biased evaluation. Objective data for complication rates such as aspiration pneumonia can provide some useful data however small sample sizes and the variability in the spectrum of clinical patients tend to preclude useful case-control studies or comparisons of surgical techniques. Other more objective measurement techniques have been utilized to attempt to compare different surgical techniques.

- *Rima Glottidis* Area (RGA)[6-9,31,35]

Measurement of RGA has been used as a surrogate marker for airflow through the larynx due to its ease of measurement in the cadaver and in the live animal. Since most surgeries are aimed at increasing the RGA, this is an objective method of classification, although has not been correlated to clinical success. Since increased airway radius will lead to increased RGA, increased RGA is thought to increase flow rates or reduce airway pressure based on the following equation:

$V=P \pi r^4/8nl$ where V is flow, P pressure, n viscosity of gas, l is length and r is radius of a tube. It is important to remember that RGA is not a direct measurement of any of the aforementioned variables.

- Laryngeal resistance[5,39] and airway pressure[42]

Two papers have examined the direct measurement of laryngeal resistance by utilizing positive pressure flow oral to the larynx in a testing chamber.[5,39] Resistance was calculated using the isovolumetric flow method, which assumes that flow is laminar. Resistance was measured at a flow of approximately 60L/min. A recent paper from the present author measured airway pressure whilst maintaining constant airflow and found that the measured airflow during that experiment was not laminar[10]. The simultaneous measurement of flow and pressure showed that by calculating laryngeal resistance by the isovolumetric method as above, that resistance changes with flow, suggesting that either deformation of the larynx is significant for the excised, paralyzed larynx, or (and) that the isovolumetric method does not apply, as expected. For that reason, the author

concluded that raw data such as pressure is a more reliable measure than resistance, as it does not require calculation based on assuming laminar or turbulent flow in the system. Since flow exists over a spectrum of laminar to turbulent, the change between them may be arbitrary.

2.8 Unilateral arytenoid lateralization techniques

UAL involved the suturing of the arytenoid cartilage to a more external cartilage to fix the position of the arytenoid. Lateralization techniques are broadly grouped into thyroarytenoid lateralization (TAL)[3] where the arytenoid is fixed laterally to the thyroid cartilage, and cricoarytenoid lateralization (CAL) [42] where the arytenoid is fixed to the caudal aspect of the cricoid cartilage. CAL and TAL procedures both cause a significant increase in RGA and alleviation of clinical signs associated with laryngeal paralysis [8,9,31,35]. *In vivo*, TAL increased RGA by a smaller proportion than CAL[8] and a cadaveric study showed similar findings[9]. It has been postulated that the combination of these two procedures (CTAL) may lead to optimal positioning of the arytenoid cartilage within the rigid structure formed by the thyroid and cricoid cartilage [3]. Comparison between CAL and CTAL in the live animal showed no significant difference between RGAs and no detectable difference in clinical signs [7]. A study in this author's laboratory determined that CTAL and CAL consistently reduced airway pressure, at airflow rates seen in normal and affected canines, compared to TAL but no significant differences were noted between CAL and CTAL[10].

The *inter-arytenoid sesamoid band* (IA band) provides a dorsal attachment between the arytenoid cartilages and prevents independent movement of the left and right arytenoids in the live animal [42,43]. It has been hypothesized that cricoarytenoid disarticulation combined with IA band transection allows dorso-lateral arytenoid movement to cause greater increase in RGA[3]. One author[9] found that sectioning the IA band caused significant reduction in the dorsoventral height of the RG but no significant increase in RGA. A recent study by the current author showed that sectioning of the interarytenoid band had no effect on airway pressure, but for a low tension TAL procedure, it did allow an increase in RGA. [10]

The cricothyroid (CTJ) joint is a firm bilateral attachment between the cricoid and thyroid cartilages that prevents the overriding of those cartilages and maintains the dorsoventral height of the *rima glottidis* during abduction. [6,43]Although CTJ disarticulation has been recommended to increase surgical exposure, its necessity has been debated as no significant increase in RGA or change in RG geometry was found [9,31] and the risk of dorsoventral collapse has been postulated with bilateral procedures [6]. This author's study found that disarticulation of the cricothyroid joint had no significant effect on airway pressure in an excised cadaver larynx. [10]

Another variable factor in arytenoid lateralization is the tension of the suture employed. It is reported that for unilateral CAL, a low suture tension resulted in a lower percentage increase in *rima glottidis* area than a high tension suture (82% and 129% increase respectively) but misalignment of the RG and epiglottis in a high tension CAL was

postulated to play a role in the increased risk of aspiration pneumonia [31]. This study did not objectively classify high and low tension and the necessity or practicality of an optimum suture tension to balance a possible risk of aspiration against increased airflow is unclear. A further study by the same group found that increasing suture tension (which included further dissection of the laryngeal specimen) on a CAL procedure did not significantly affect laryngeal resistance with an open glottis used as a measure of maximum airflow. However higher suture tension reduced closed glottal resistance, which was used as a measure of the fit of the RG and epiglottis, and was predicted to increase the risk of aspiration pneumonia *in vivo*[5]. This has not been investigated or confirmed by any clinical study. A previous study by the present author showed that increasing tension without an increase in dissection significantly reduced airway pressure for TAL at 30 L/ min, CAL at 45 to 120 L/min, which incorporates resting flow rates recorded for both normal animals with and those with iLP. [10]

The RG is considered to be the most important resistor to airflow, and is the therapeutic target for laryngeal surgeries. CAL procedures in the canine cadaver larynx result in significantly reduced airway pressure compared to the TAL procedure[8,10,35]. At consistent levels of dissection and suture tension (100g), a significant increase in RGA (114.8% to 152.5% increase in RGA) was seen after sectioning of the IA band, however no significant reduction in airway pressure was seen. It is unknown why an increase in RGA would not result in reduced airway pressure but the effects of the TAL and CAL suture have not been examined with regard to 3D cross sectional imaging of the larynx. The TAL, CAL, and CTAL procedures had different effects on airway pressure and

may have altered airflow by a more complex mechanism than simply by increasing the RGA. The RGA was not a reliable correlate of airway pressure in that study[10].

2.9 Computed Tomography of the larynx

Computed tomography (CT) of the larynx is commonly used in humans in the pre and post therapeutic work-up for neoplastic conditions[44] and has been used to produce computerized 3D models for assessing fluid flow and particle deposition through the trachea and larynx. More recently a veterinary study used CT to classify upper airway obstruction including iLP[12]. No study has examined the measurable volume of the larynx and it is currently unknown what effect surgical manipulation of the arytenoid cartilages may have on structures other than the RG. The volume of air contained within the larynx is measurable on CT using proprietary software (Osirix©, Osirix foundation, Geneva) and CT can be performed with patients under minor sedation at minimal risk and therefore provides a non-invasive and objective tool for anatomical assessment. Only one other study has utilized CT in assessing the normal anatomy of the canine larynx[11].

2.10 Conclusions regarding Unilateral Arytenoid Lateralization surgery for the idiopathic Laryngeal Paralysis patient

Ultimately all surgical options except the nerve-muscle pedicle graft have aimed to increase the size of the RG, whether by directly removing tissue, or fixing the arytenoid cartilage out of the path of airflow. UAL has been suggested to cause mismatch between the RG and epiglottis in dogs that may have significant esophageal disease and

swallowing dysfunction, possibly predisposing to aspiration pneumonia preoperatively and for the rest of their lives post-operatively. The decision of how far to lateralize the arytenoid cartilage is a subjective decision made by the surgeon, with no published clinical outcome studies available. Given that often the underlying factors responsible for aspiration cannot be reversed and a patient's life can be permanently affected by this decision, more objective assessment of the UAL procedure is required.

2.11 Rationale for this study

Arytenoid lateralization is a technique by which the RG is enlarged by an amount subjectively assessed by the operating surgeon to allow sufficient airflow to prevent clinically relevant dyspnea without significantly increasing the risk of aspiration pneumonia in dogs that already have esophageal disease or have a predisposition for developing it.[30] *In vitro* evidence exists that high tension CAL procedures lead to greater reduction in airway pressure at clinically relevant flow rates [10] as well as increased RGA and a postulated mismatch between the epiglottis and glottis that may allow increased aspiration of fluid or food into the lower airways[31]. Clinical studies have failed to demonstrate differences in outcome related to technique and currently there is no validated method to measure laryngeal airway pressure or laryngeal resistance, or the mismatch of the epiglottis and glottis in the live patient. Variability of surgical technique between surgeons is an unknown factor in arytenoid lateralization and may result in a heterogeneous treatment effect of patients subject to arytenoid lateralization.

To allow the prospective assessment of clinical patients, a desirable measurement of the treatment effect of arytenoid lateralization would be non-invasive, in that full anesthesia and surgical measures would not be required, objective in that it could be measured consistently with low number of patients and would correspond directly to lateralization of the arytenoid and/or indirectly to increases in RGA or mismatch of the epiglottis and glottis. CT may offer the ability to examine the displacement of the arytenoid cartilage within the rigid 3 dimensional structures formed by the overlapping cricoid and thyroid cartilages. The ability to classify the amount of lateralization objectively and correlate the 'surgical dose' with outcome or complication rates could allow the standardization of surgical treatment for iLP and subsequently its optimization by well-designed prospective assessment.

2.12 Overall goals of this study

- Assess the feasibility of CT for measuring arytenoid displacement in arytenoid lateralization.

- Identify potential CT measures of arytenoid lateralization for future prospective assessment in clinical cases

2.13 Null hypotheses

1. Computed tomography of the excised canine cadaver larynx will be unable to detect the arytenoid cartilages as a separate entity within the larynx and their change in position due to arytenoid lateralization surgery.

2. No significant differences will be seen between measurements made after dissection of the larynx (prior to suture tightening) and the end of the experiment, suggesting that sequential tensioning of sutures in this experiment deform the larynx and invalidate sequential measures.

3. No significant differences will be seen due to the effects of surgical techniques and tension and no suitable CT measurement parameter, which might warrant further prospective evaluation in clinical studies, will be identified.

3. MATERIALS AND METHODS

3.1   Specimens

Eight laryngeal specimens were obtained from young adult large breed shelter dogs of similar body size (weight varied from 16.9 kg to 30.3 kg dependent on body condition; Mean bodyweight 22.71 kg), which were euthanized by IV solution of a pentobarbital-phenytoin solution for reasons other than upper respiratory disease. The dog's weights are shown in table 1.

Table 1. Dog weight

A table to show the weight of the dogs used in this study.

Dog ID	Body Weight (Kg)
1	20.9
2	19.0
3	22.0
4	16.9
5	24.9
6	30.3
7	22.8
8	24.9

Each specimen comprised of the larynx, caudal oropharynx, proximal esophagus and proximal 5 tracheal rings. All larynges were examined to ensure that they were anatomically normal. Data collection was performed within 24 hours of tissue harvesting. Excess cervical tissue was excised and the esophagus was incised dorsally to permit visualization of the RG. Each larynx was secured to an acrylic board (Optix,

Eden Praire, Mn) using a polyvinyl chloride attachment (Ryan Herco Flow Solutions, Ca) at the base of the epiglottis and the third tracheal ring. The epiglottis was allowed to rest in a neutral position and was not pulled cranially. This allowed stabilization of the larynx without impeding arytenoid motion or the use of radio-opaque materials, which might cause CT artefact. Fiducial markers (Beekley Corporation, Bristol, CT) were secured at the base of the RG and at the most caudal point of the cricoid cartilage (Gluture, Abbott Animal Health, Il) to establish the laryngeal axis. The prepared laryngeal specimen is shown in figure 1.
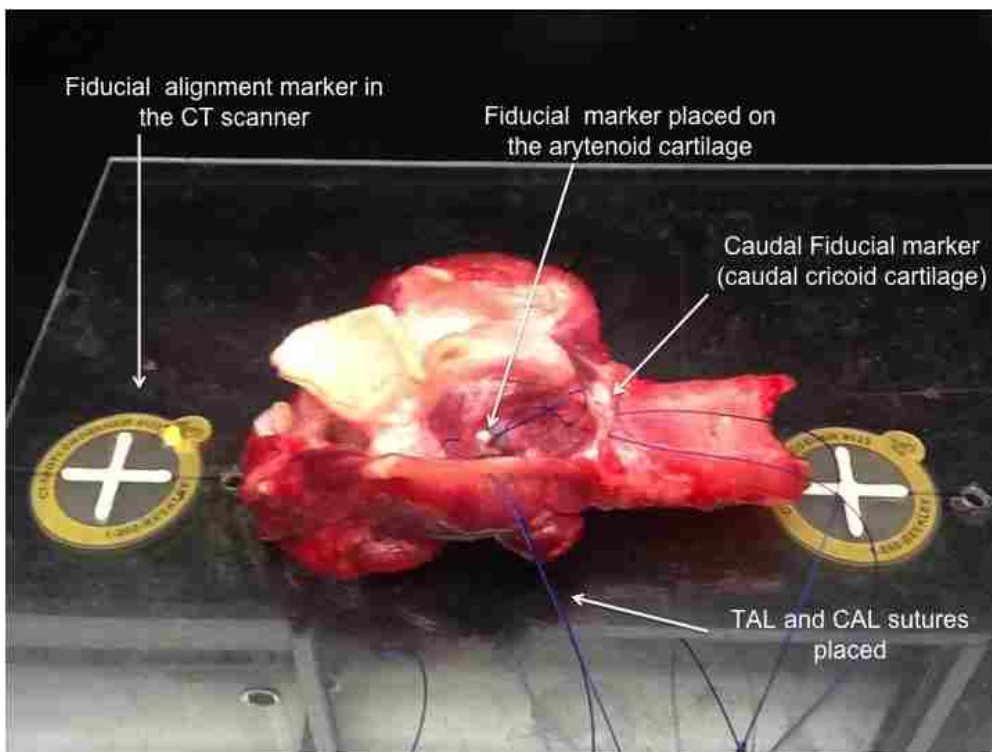
Figure 1. The prepared laryngeal specimen

Figure 1 shows the prepared canine cadaver larynx. The larynx has been mounted on an acrylic board with two PVC attachments. The acrylic board contains markers to allow orientation of true cranio-caudal alignment of the specimen. This specimen has had the

TAL and CAL suture placed as in Griffiths et al.[8] and fiducial markers placed as illustrated in Fig 2.

3.2 Laryngeal specimen preparation

All procedures were performed on the left arytenoid cartilage by a single surgeon (JW) as described in a previous study[10]. Thyroarytenoid lateralization (TAL) and cricoarytenoid lateralization (CAL) sutures were placed using 2-0 polypropylene (Prolene, Ethicon, Blue Ash, Ohio) as described previously[8] but the cricothyroid articulation and inter-arytenoid band was left intact. Sutures were passed through the ventral portion of the articular surface of the muscular process of the arytenoid cartilage, which has been shown to have the greatest cartilage thickness[45]. Sutures were tensioned using a tensionmeter (Berkley 50lb Digital Fish Scale, Pure fishing inc., Spirit lake, IA) using 100g (a tension that caused fixing of the arytenoid with minimal abduction) and 500g (a significant level of abduction without risking arytenoid fracture or pull through), as in previous studies[10], and the sutures were secured with a fluid administration set clamp (Churchill medical systems, Lansdale, PA) which was radiolucent and maintained abduction during measurement but permitted the same suture to be used in sequential interventions. Figure 2 shows the position of the fiducial markers on the lateral projection achieved from a Maximal Intensity Projection (MIP) a volume rendering method used in 3D reconstruction of the larynx.
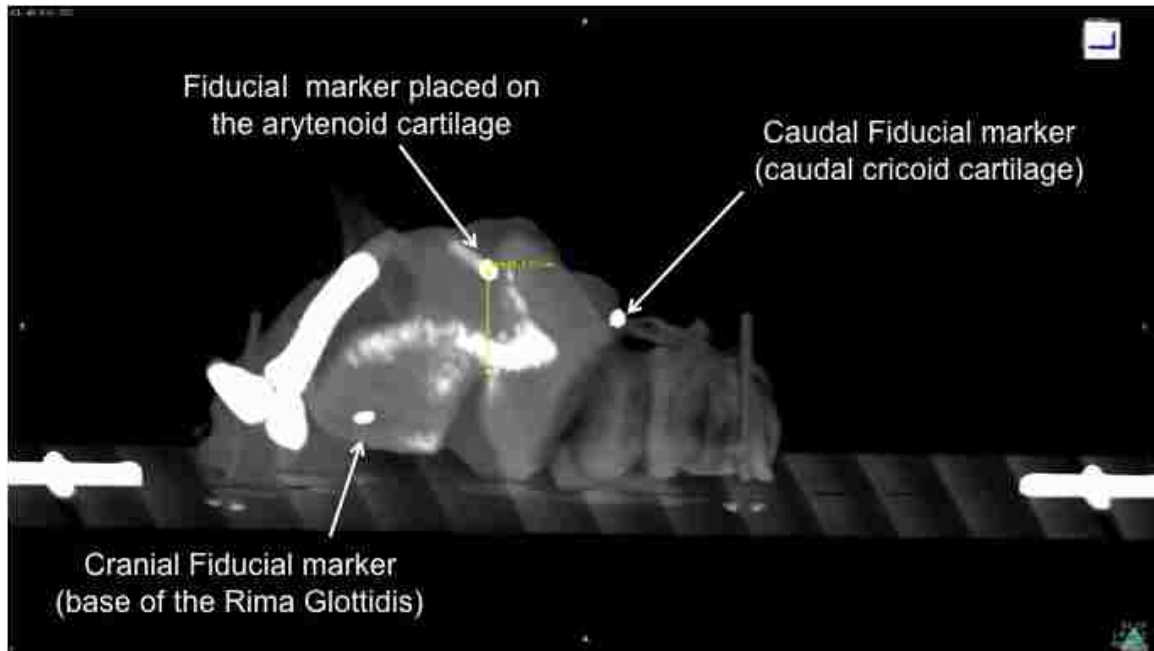
Fig 2. Fiducial marker placement illustrated on MIP 3D reconstruction
This figure shows a lateral projection of the larynx, taken from the 3D MIP reconstruction of the larynx depicted in Fig 1. This illustrates the placement of the fiducial markers. The cranial fiducial marker is placed at the base of the *rima glottidis*. The caudal marker is placed at the most medial, dorsal and caudal aspect of the cricoid cartilage.

3.3 Computed Tomography

Computed tomography (CT) (GE Lightspeed 16-slice) was performed with 0.6mm transverse slice thickness for each specimen before intervention (presurgical) and after dissection and suture placement of TAL and CAL sutures and a fiduciary marker fixed with cyanoacrylate glue (Gluture, Abbott Animal Health, Il) to the muscular process of the arytenoid cartilage (this data was recorded as 'dissected'). CT was performed for TAL, CAL and CTAL at 100g tension, which was found to cause minimal abduction and 500g tension which was used for high tension in a previous study[10]. For CTAL, the CAL suture was tightened prior to the TAL suture as previously reported[7]. Procedures

were performed in a random order dictated by generation of a random number between 0 and 1 for each procedure using a proprietary website (www.random.org). Arytenoid lateralization procedures were performed based on the descending order of random number. After the last arytenoid lateralization procedure was performed, the sutures were loosened and CT was repeated (this data was recorded as postsurgical).

 Measurement of laryngeal volumes and fiducial marker measurement were measured solely by one operator for each measure (JW or NR) who was blinded to the suture type and tension employed, however blinding for the presence of the suture fastening clamp could not be achieved. Volume was calculated by measuring the cross sectional area of the larynx on each axial slice and using the volume measurement tool of Osirix© v. 3.6.1. (Osirix foundation, Geneva) Volume was measured on the soft tissue window, to replicate most closely the parameters that would be used for the live animal and minimize edge effect. 3D reconstructions using the same software application were performed in order to allow orientation of each laryngeal specimen along a conserved axis made by the superimposition of the cranial and caudal fiducial markers. From this axis horizontal and vertical displacement of the arytenoid cartilage marker, and measurement derivations thereof could be objectively assessed. Reconstructions were also positioned to allow a conserved rostral projection to allow measurements to assess the distance between left and right arytenoid cartilages (Inter-arytenoid distance), left arytenoid cartilage and thyroid cartilage (Arytenoid thyroid wing distance, ATW).

3.4 Computed Tomographic measures of Laryngeal volume

3.4.1 Laryngeal volume

The internal volume of the larynx was measured between the most rostral axial slice with dorsal coverage by the interarytenoid band, as illustrated in figure 3a, to the most caudal axial slice containing the caudal fiducial marker, as shown in figure 3b. The volume was calculated using the Osirix measurement tool, after manual tracing the cross sectional area of air contained within the larynx at each 0.6mm axial slice between the rostral and caudal limits of measurement.
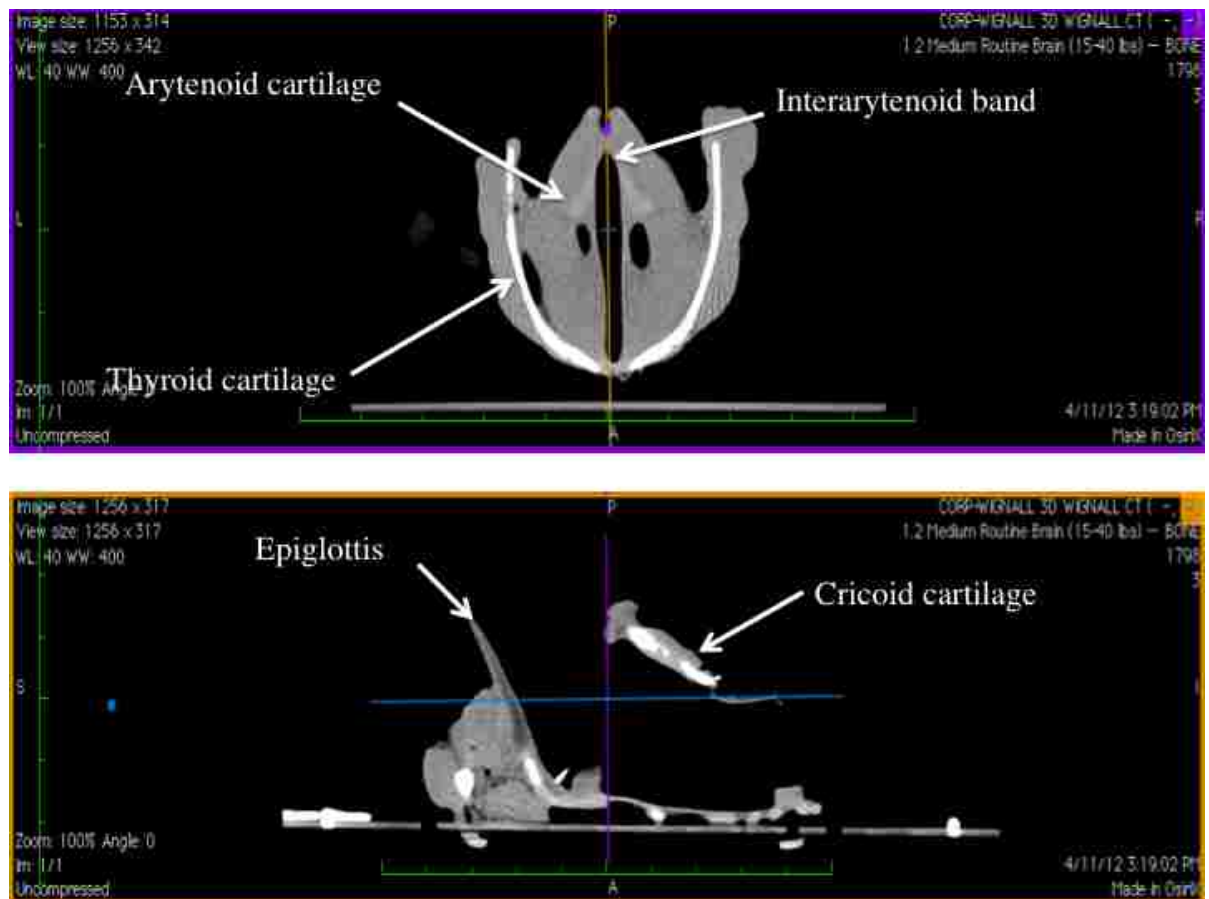
Fig 3a. Laryngeal Volume, cranial extent
Fig 3a shows an axial (above) and lateral (below) multiplanar reconstruction of the larynx. The cursor rests on the most cranial point of measurement for volume. This is the most cranial axial slice with complete dorsal coverage.
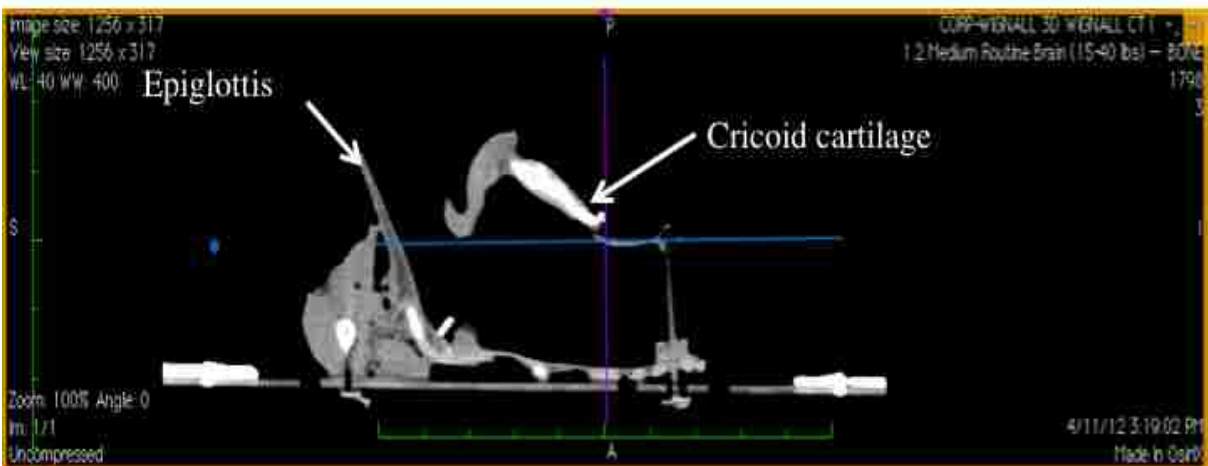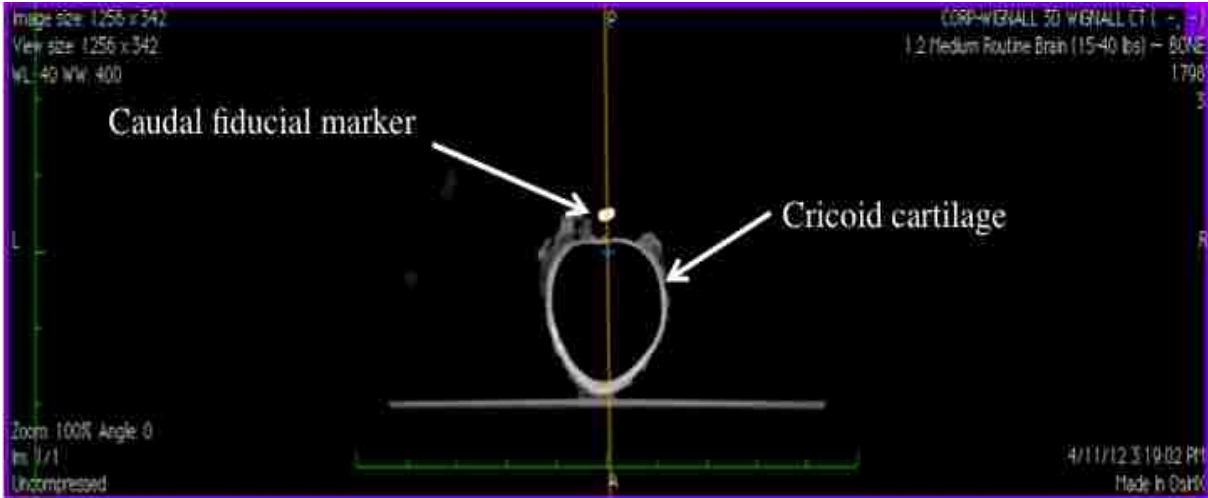
Fig 3b. Laryngeal volume, caudal extent
This figure shows an axial (above) and lateral (below) multiplanar. The cursor rests on the most caudal point of measurement for volume. This is the most caudal axial slice with a complete dorsal coverage of cartilage (caudal cricoid cartilage).

3.4.2. Central to rostral laryngeal cross sectional area; cCSA

Central to rostral laryngeal cross sectional area (cCSA) was measured on the MIP

setting incorporating a 1.8mm rostral slice which contained both the cranial fiducial

marker and dorsal coverage provided by the interarytenoid band. The single displayed

cross sectional area of air was traced and the area calculated using the Osirix© v. 3.6.1.
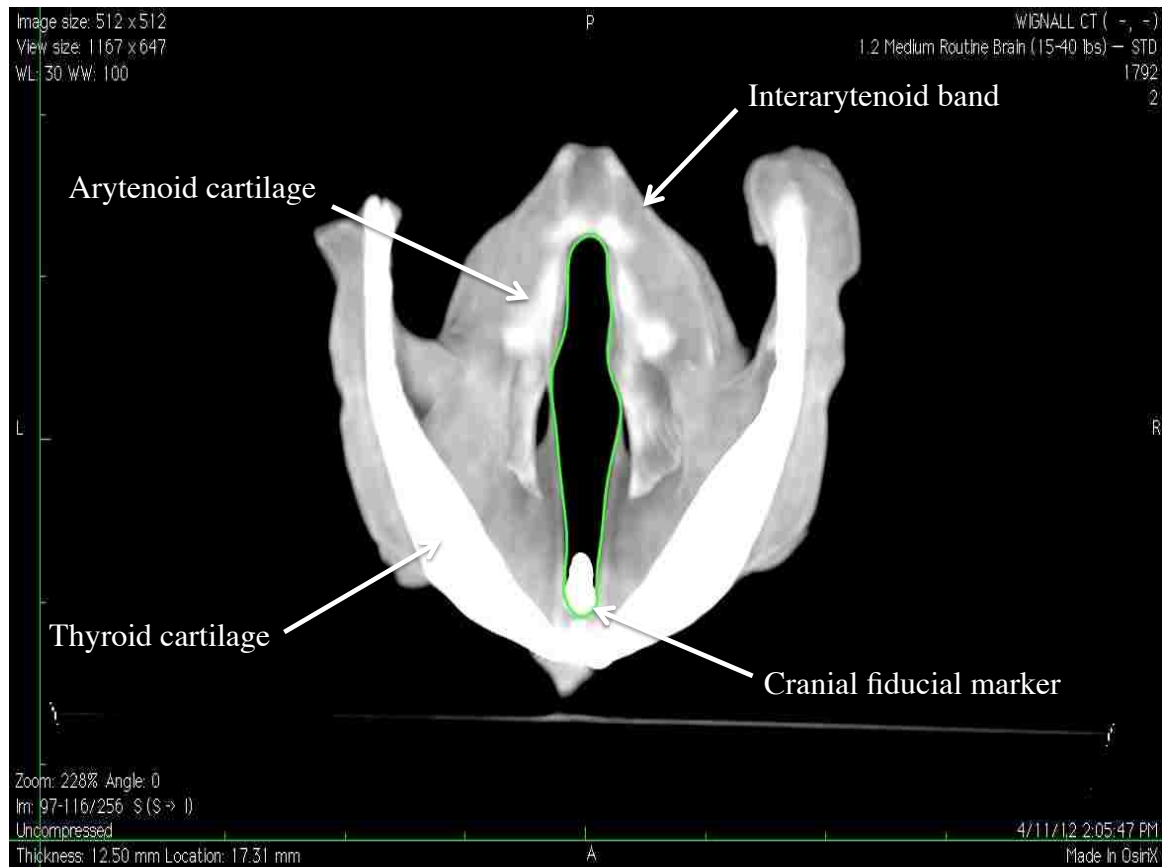
Fig 4. Central to rostral cross sectional area; **cCSA**
This axial MIP CT slice illustrated the measurement of cCSA. The cross sectional area
of the airway was measured at the most rostral axial MIP slice (incorporating 1.8mm of
tissue), which included the cranial fiducial marker and the interarytenoid sesamoid band.
The green tracing shows the outline of the area measured.

3.4.3 Conserved laryngeal axis length; CLA

The lateral projection of the 3D MIP reconstruction was aligned by superimposition of

the thyroid cartilages. A line (conserved laryngeal axis, CLA) was projected which

passes through the center of the cranial and caudal fiducial markers. The length between

the center of the cranial and caudal fiducial markers was measured as CLA.
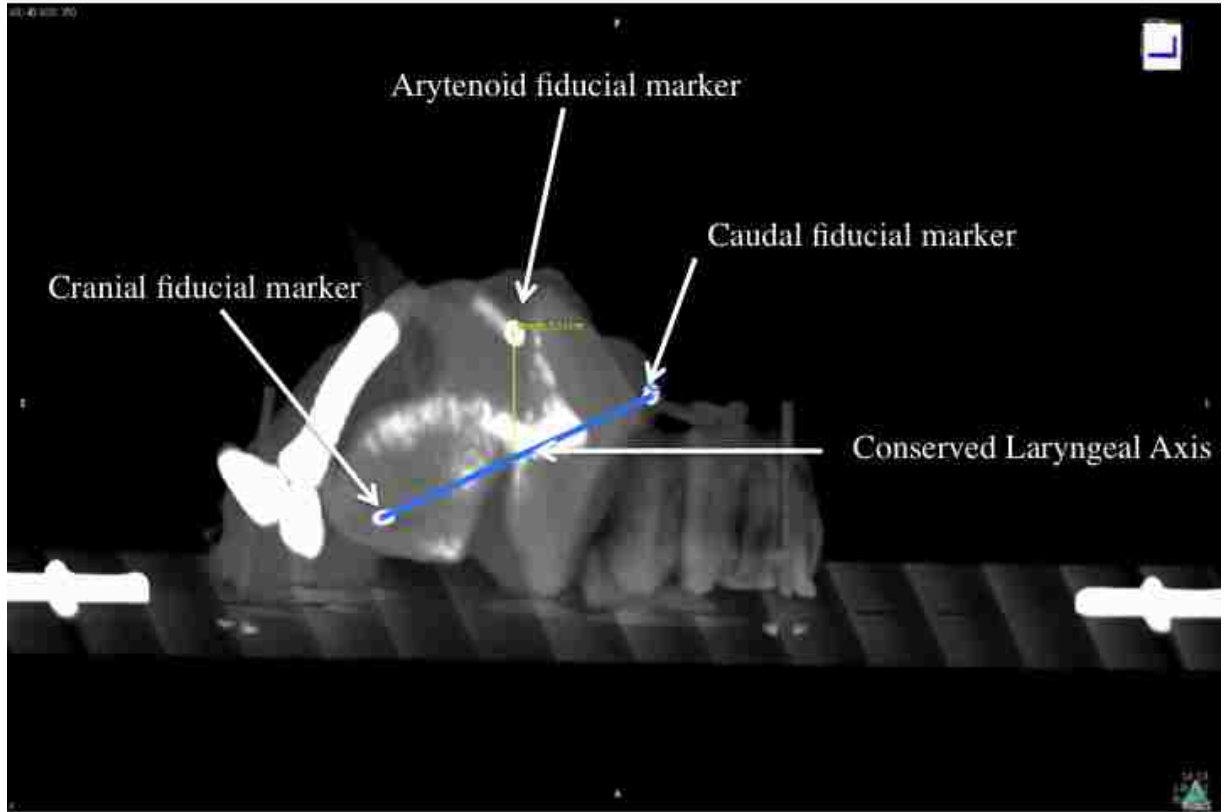
Fig 5. Conserved laryngeal axis length; CLA
This lateral projection, taken from the 3D MIP reconstruction, illustrates the measurement of the laryngeal axis. The solid blue line represents the conserved laryngeal axis (CLA), which is the distance between the cranial and caudal fiducial markers.

3.4.4 Horizontal displacement of the arytenoid fiducial marker; fid horiz

The dorsoventral projection of the 3D MIP reconstruction was used to assess horizontal

arytenoid displacement from midline. The laryngeal axis was aligned end on to ensure a

constant and repeatable positioning of the larynx. It was then rotated to ensure

symmetrical positioning. Horizontal displacement of the arytenoid was measured by

the distance between the central axis of the larynx and the fiducial marker placed on the

muscular process of the arytenoid cartilage.
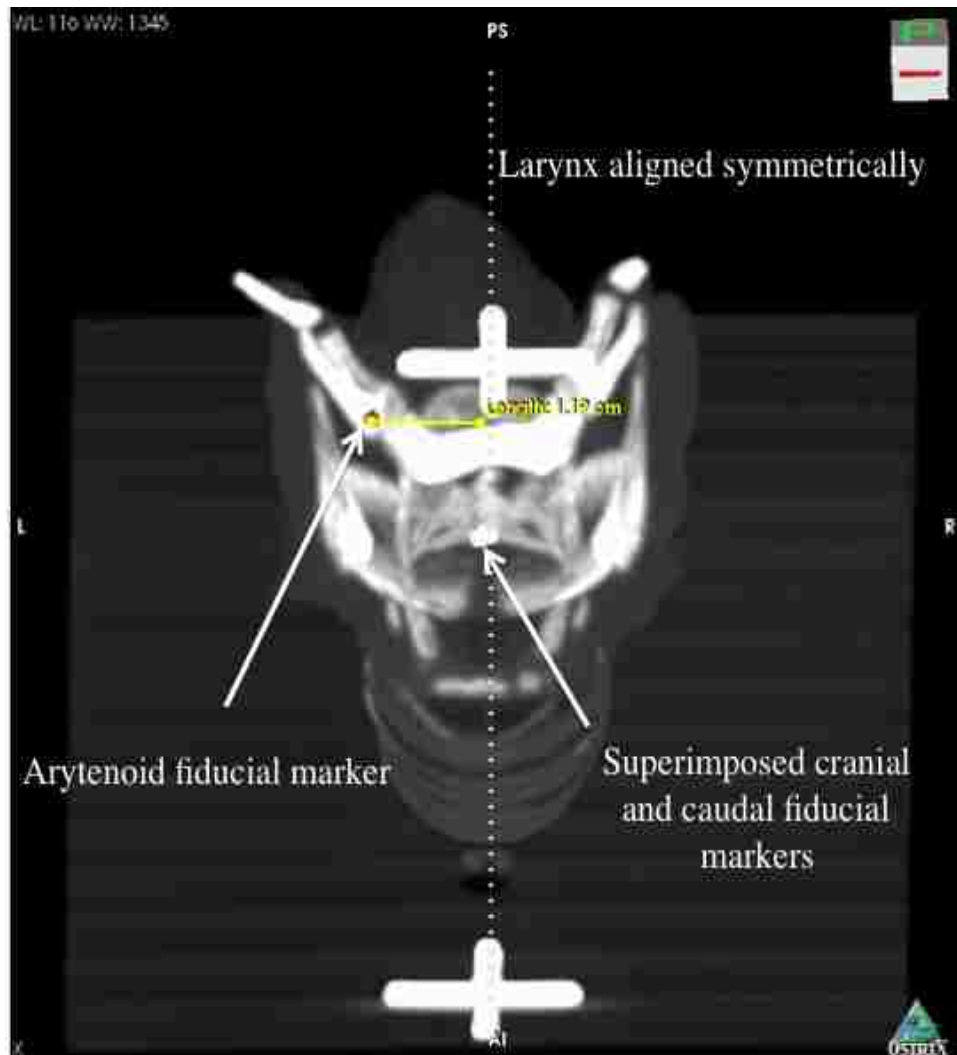
Fig 6. Horizontal fiducial marker displacement; fid horiz
This dorsoventral projection was taken from the 3D MIP reconstruction was used to assess horizontal arytenoid motion. The cranial and caudal fiducial markers were superimposed to ensure a constant and repeatable positioning of the larynx. The larynx was then rotated to ensure it was symmetrically aligned around the positioning markers. Horizontal displacement of the arytenoid was measured by the distance between the central axis of the larynx and the fiducial marker previously placed on the muscular process of the arytenoid cartilage.

3.4.5 Vertical displacement of the arytenoid fiducial marker; fid vert

The lateral projection of the 3D MIP reconstruction was used to position the larynx in true lateral based on a superimposition of the thyroid cartilages. The CLA was established and vertical displacement of the arytenoid was measured by the distance between the CLA of the larynx and the fiducial marker placed on the muscular process of the arytenoid cartilage. The fid vert measurement is illustrated in Fig 7.
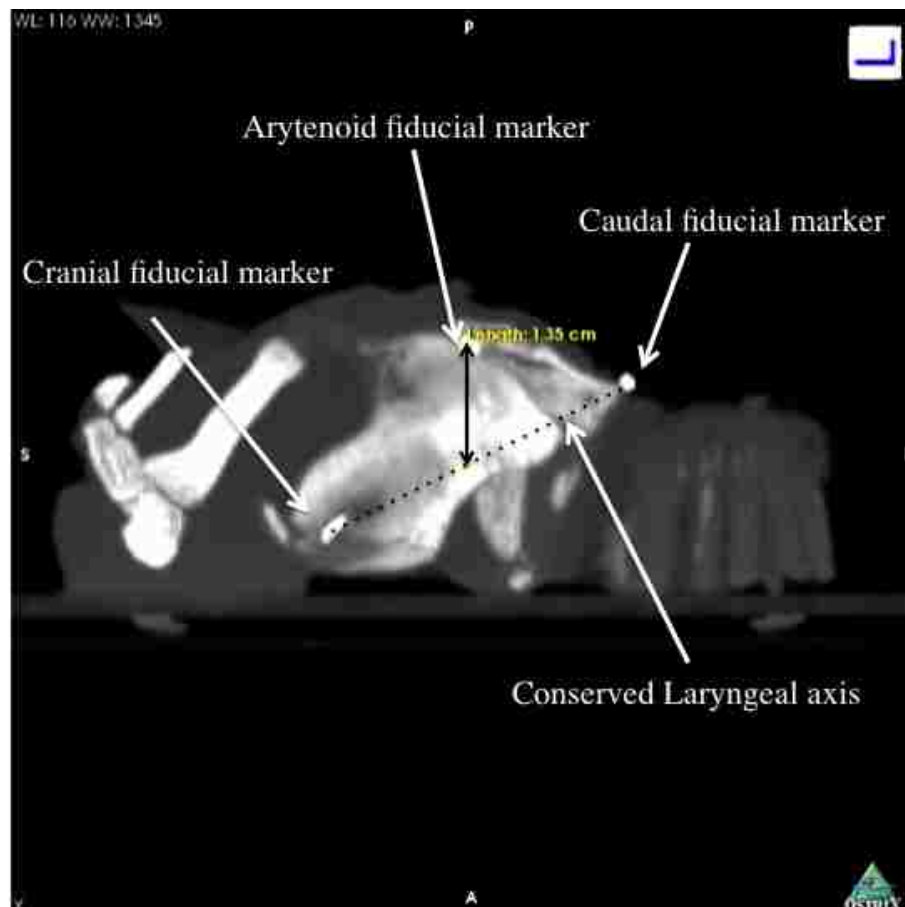
Fig 7. Vertical fiducial marker displacement; fid vert
This lateral projection was taken from the 3D MIP reconstruction and used to position the larynx in true lateral based on superimposition of the thyroid cartilages. The laryngeal axis was established and vertical motion of the arytenoid was measured by the vertical distance between the conserved laryngeal axis and the arytenoid fiducial marker. The measured distance is annotated with a solid black double-ended arrow.

3.4.6 Caudal arytenoid displacement; CauAM

The lateral projection of the 3D MIP reconstruction was used to position the larynx in true lateral based on a superimposition of the thyroid cartilages. Caudal arytenoid displacement was measured between the intercept point of the CLA and Fid vert measurements (see Fig 7) to the most caudal point of the CLA. The CauAM measurement is illustrated in figure 8.
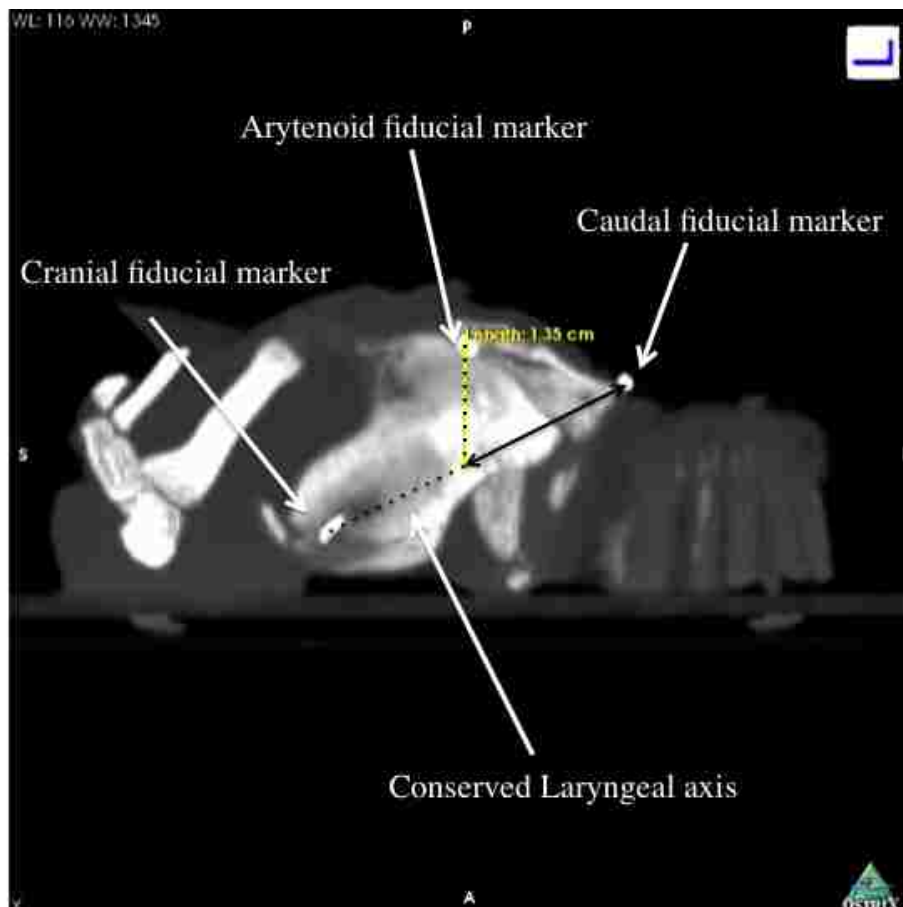
Fig 8. Caudal arytenoid displacement; CauAM
The lateral projection of the 3D MIP reconstruction was used to position the larynx in true lateral based on an overlap of the thyroid cartilages. The conserved laryngeal axis (CLA) was established and caudal arytenoid motion (CauAM) was the measured distance on the CLA between the point where the Fid vert measurement line contacted the CLA and the caudal fiducial marker. The measured distance is annotated with a solid black double-ended arrow.

3.4.7 Arytenoid to thyroid wing distance; ATW

The 3D MIP reconstruction in a mixed soft tissue window (WL 168; WW 200) was oriented to show the rostral view of the larynx. The distance between the inner surface of the arytenoid cartilage and the center of the wing of thyroid cartilage was measured as the arytenoid to thyroid wing distance (ATW) and is shown in Fig 9.
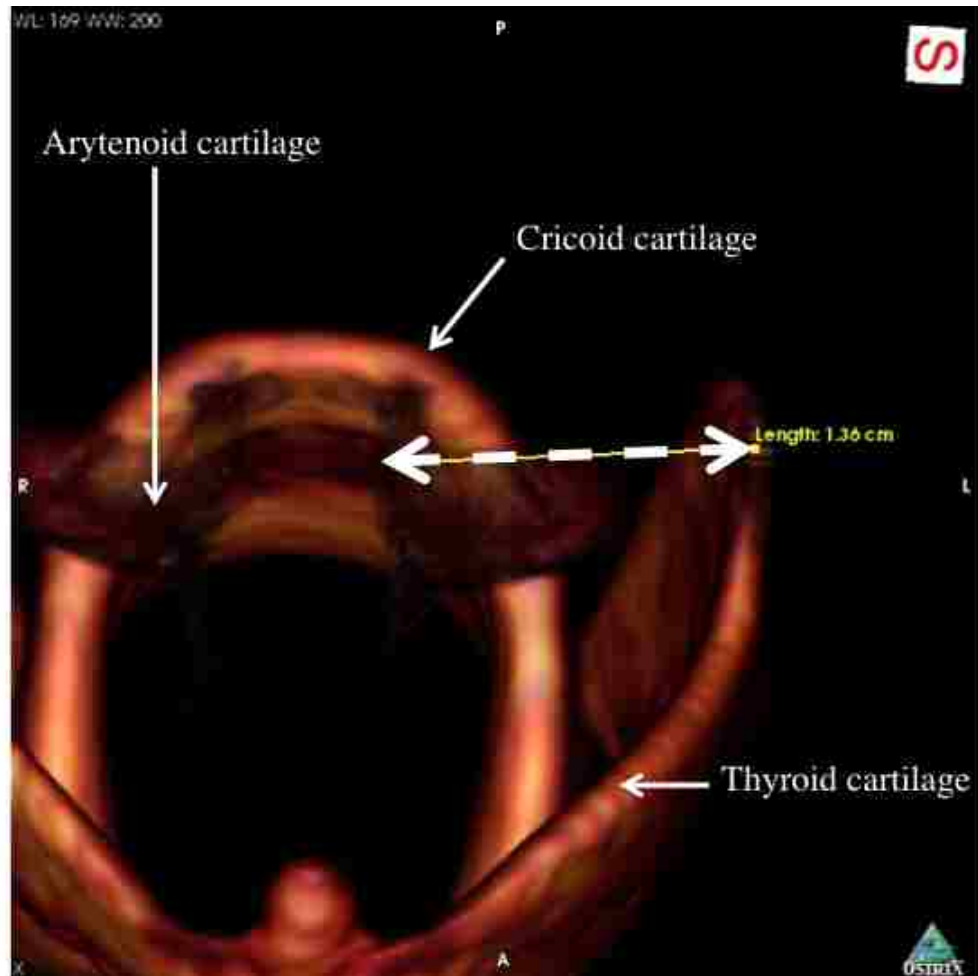
Fig 9. Arytenoid to external thyroid wing; ATW
3D MIP reconstruction in a mixed soft tissue window (WL 168; WW 200) was oriented to show the rostral view of the larynx. The distance between the inner surface of the arytenoid cartilage and the wing of thyroid cartilage was measured as ATW. The measured distance is annotated with a dashed white line with a double-ended arrow.

3.4.8 Interarytenoid distance; IAD

The 3D MIP reconstruction in a mixed soft tissue window (WL 168; WW 200) was oriented to show the rostral view of the larynx. The distance between the most medial portions of the arytenoid cartilages was measured as inter arytenoid distance (IAD) and is shown on figure 10.
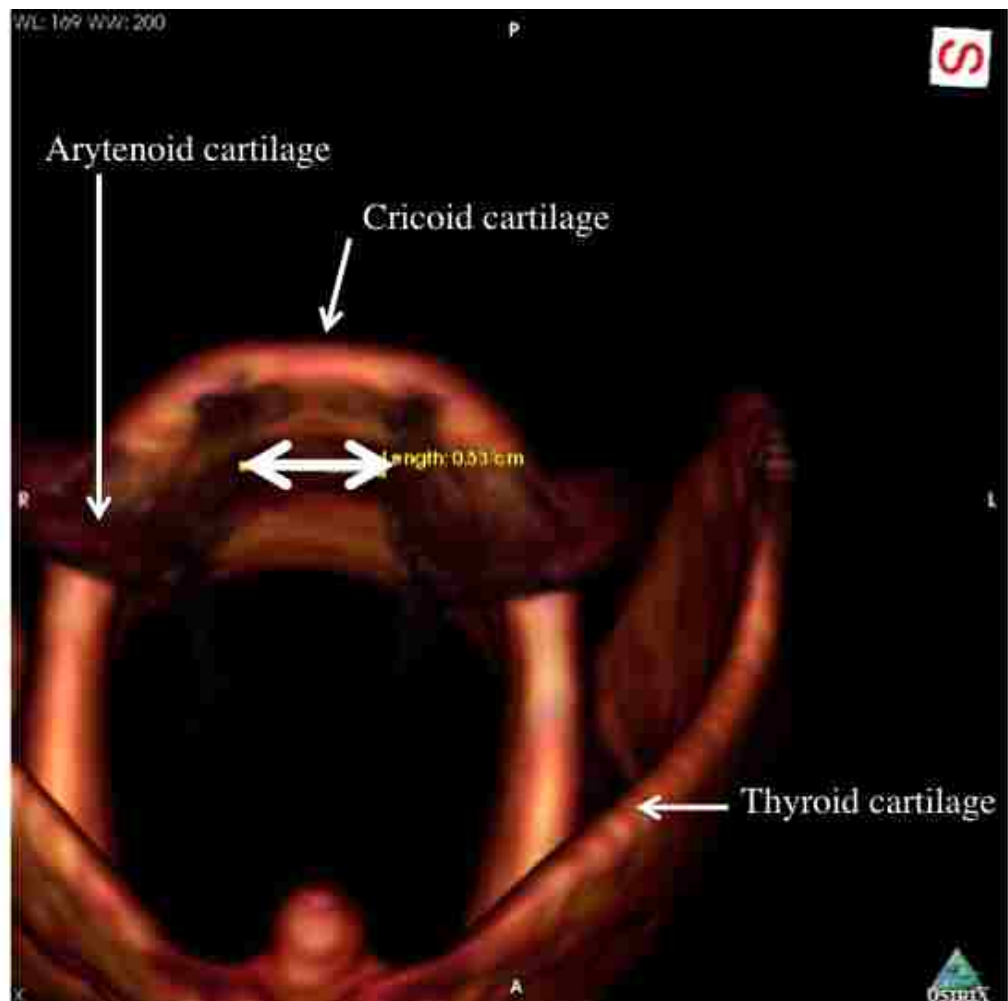
Fig 10. Interarytenoid distance, IAD
3D MIP reconstruction in the soft tissue window was oriented to show the rostral view of the larynx. The distance between the inner surfaces of the arytenoid cartilages was measured as Interarytenoid distance. The measured distance is annotated with a solid white line with a double-ended arrow.

3.5 Statistical analysis

One-way analysis of variance with fixed effects (surgical procedure and order of procedure) was performed to identify overall significance. Significant differences between measured CT parameters for presurgical, dissected and postsurgical data as well as different arytenoid lateralization techniques and suture tensions were assessed by the least square means procedure and pairwise differences were assessed using a Tukey adjustment for type 1 error control. Each observation was normalized as a percentage of the presurgical parameter if available or dissected parameter (for fiducial marker measurements) for each larynx. The assumptions of the independence of observations, their normality, and homoscedasticity were assessed. Significant associations were assessed with the Shapiro-Wilk test for departures for normality. Dissected and post surgical data was assessed for significant associations by assessment of a Kernel distribution of bootstrapped means. Open source statistical software was used throughout (R Trick or Treat version 2.15.2, Vienna, Austria).

4. RESULTS

All CT scans were able to distinguish the arytenoid cartilage from the thyroid and cricoid cartilage. CT scans of unaltered larynges were inspected by one author (JW) and assessed for abnormal anatomy. All specimens were assessed to be within normal limits. All CT scans were deemed diagnostic and no data points were discarded.

4.1 Laryngeal Volume

Figure 11 shows the normality plot for laryngeal volume data.
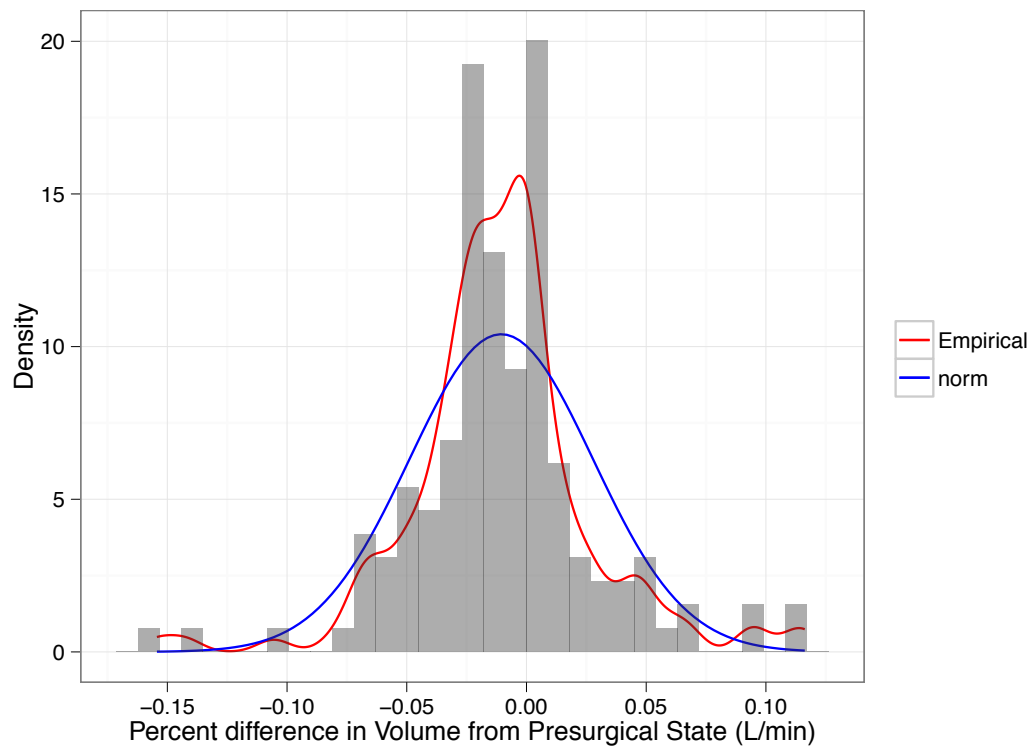
Fig 11. Laryngeal Volume, normality plot
The normality plot for volume data for the 8 dogs used in the study is shown in fig. 11.
Visual assessment suggests no departure from normality.

In general, laryngeal volume was greatest prior to surgery, as shown in fig 12. All surgical interventions caused a reduction in volume however these reductions were not significant. Surgical manipulation of the larynx by all arytenoid lateralization procedures had no significant effect on laryngeal volume. Mean and standard deviation is shown in table 2 and the box and whiskers plot in figure 12.
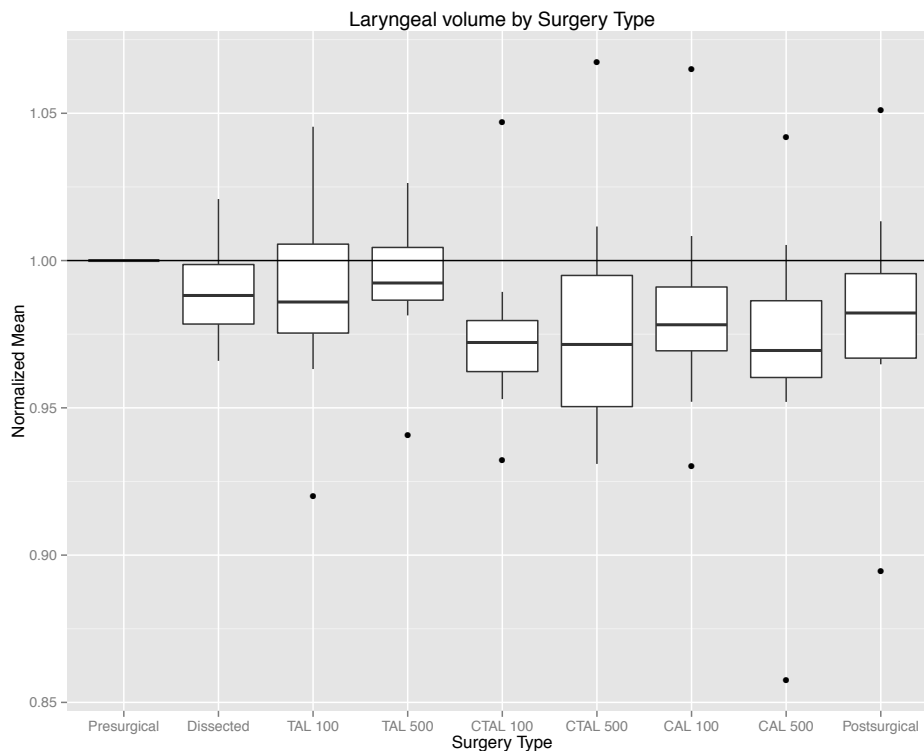
Fig 12. Laryngeal volume, box and whiskers plot

This figure shows the box and whiskers plot for the normalized means of laryngeal volume by surgery type. No significant differences are noted between presurgical values (normalized to 1.00) and different surgery types. No significant differences are noted between surgery types.

Table 2. Laryngeal volume, mean+-SD, pairwise comparisons

This table shows the mean and standard deviation for Laryngeal Volume and the p values for pairwise comparison with the presurgical value ($p < 0.05$ is taken as significant) i.e. how does surgery affect laryngeal volume.

Surgical Intervention		
Means	**Laryngeal Volume (cm^3)**	**p value, pairwise comparison to presurgical**
Presurgical	7.33	
Dissected	7.24	
TAL100	7.19	1.00
TAL500	7.24	1.00
CAL100	7.18	1.00
CAL 500	7.12	1.00
CTAL 100	7.13	1.00
CTAL 500	7.15	1.00
Postsurgical	7.15	1.00
SD	**Laryngeal Volume (cm^3)**	
Presurgical	1.93	
Dissected	1.84	
TAL100	1.62	
TAL500	1.71	
CAL100	1.71	
CAL 500	1.96	
CTAL 100	1.73	
CTAL 500	1.67	
Postsurgical	1.63	

4.2 Central to rostral laryngeal cross sectional area (cCSA)

Table 3 shows the mean and standard deviation for cCSA. All surgeries increase cCSA compared to presurgical however this is not a significant increase. CAL procedures followed by CTAL followed by TAL procedures show the greatest increase compared to presurgical values. Increasing tension from 100g to 500g increased cCSA for all procedures however this was not significant. The box and whisker plot shown in figure 13 shows data normalized to presurgical values.

Table 3. cCSA, mean+-SD, pairwise comparisons
This table shows the mean and standard deviation for Central cross sectional area (cCSA) and the p values for pairwise comparison to presurgical values (p<0.05 is taken as significant). No significant changes are seen.

Surgical Intervention	MIP projection	
Means	**Central CSA (cm^2)**	**p value, pairwise comparison to presurgical**
Presurgical	0.81	
Dissected	0.80	
TAL100	0.86	1.00
TAL500	0.87	1.00
CAL100	0.94	1.00
CAL 500	0.99	1.00
CTAL 100	0.92	1.00
CTAL 500	0.93	1.00
Postsurgical	0.68	1.00
SD	**Central CSA (cm^2)**	
Presurgical	0.29	
Dissected	0.33	
TAL100	0.32	
TAL500	0.36	
CAL100	0.32	
CAL 500	0.34	
CTAL 100	0.29	
CTAL 500	0.40	
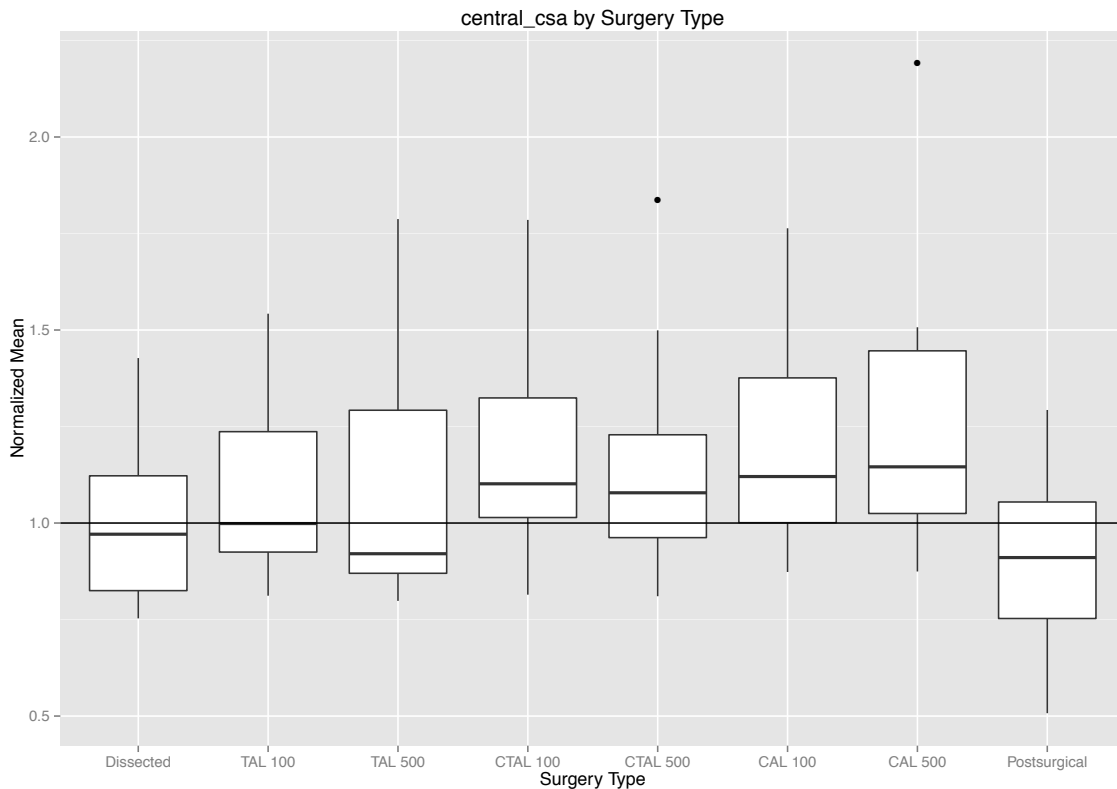
Fig 13. cCSA, box and whiskers plot
This figure shows the box and whiskers plot for the normalized means of central to rostral cross sectional area (cCSA). No significant differences are noted between presurgical values (normalized to 1.0) and different surgery types. No significant differences are noted between surgery types.

4.3 Horizontal displacement of the arytenoid fiducial marker

Fid horiz is greatest for CTAL procedures followed by CAL and TAL. There is a weak

trend for suture tension increasing displacement however no significant associations are

seen. No comparison can be made with presurgical values since no fiducial marker may

be placed prior to surgery. The Fid horiz data is illustrated in table 4 and fig 14 is a box

and whisker plot normalized to dissected data. Table 5 shows the p values for pairwise

comparisons between procedures with no significant differences seen. Figure 15 shows

a representative MIP reconstruction view of a larynx. The lines shown for each surgical

procedure are scaled to represent the mean horizontal displacement of the arytenoid

fiducial marker (fid horiz).

Table 4. fid horiz and fid vert, mean+-SD, pairwise comparisons
This table shows the mean and standard deviation for horizontal (Fid Horizontal) and
vertical (Fid Vertical) motion of the arytenoid fiducial marker.

Surgical Intervention	3D reconstruction	
Means	**Fid Horizontal (mm)**	**Fid Vertical (mm)**
TAL100	13.19	13.01
TAL500	12.90	12.81
CAL100	12.14	13.50
CAL 500	12.40	12.91
CTAL 100	12.94	12.83
CTAL 500	13.23	12.68
Postsurgical	13.06	13.78
SD	**Fid Horizontal (mm)**	**Fid Vertical (mm)**
Dissected	1.17	0.86
TAL100	1.72	1.14
TAL500	1.79	1.23
CAL100	1.15	1.45
CAL 500	0.86	1.48
CTAL 100	1.27	1.02
CTAL 500	1.29	0.88
Postsurgical	2.04	1.14
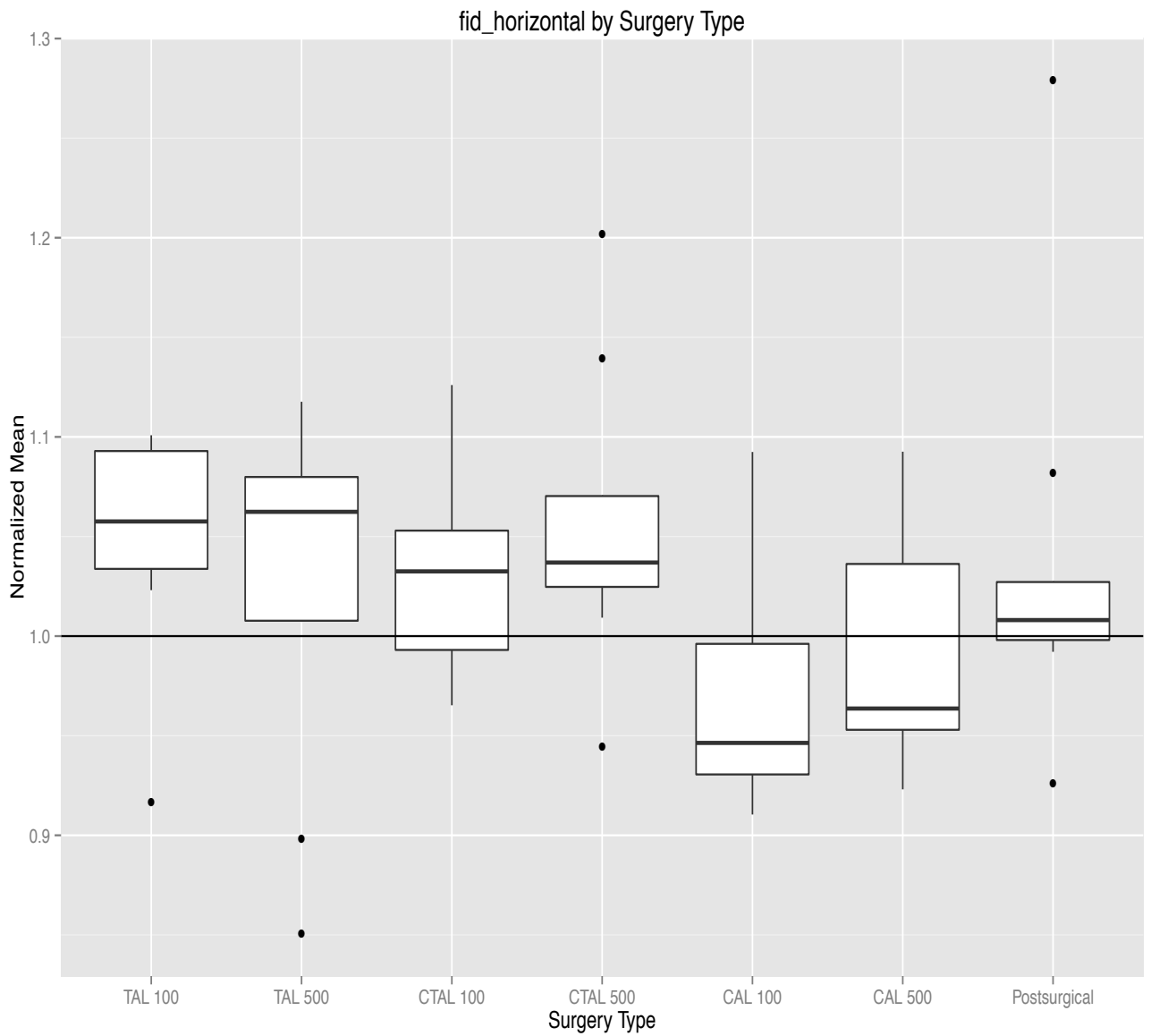
Fig 14. fid horiz, box and whiskers plot
This figure shows the box and whiskers plot for the normalized means of Horizontal motion of the arytenoid fiducial marker (Fid Horizontal) by surgical technique. No significant differences are noted between different surgery types.
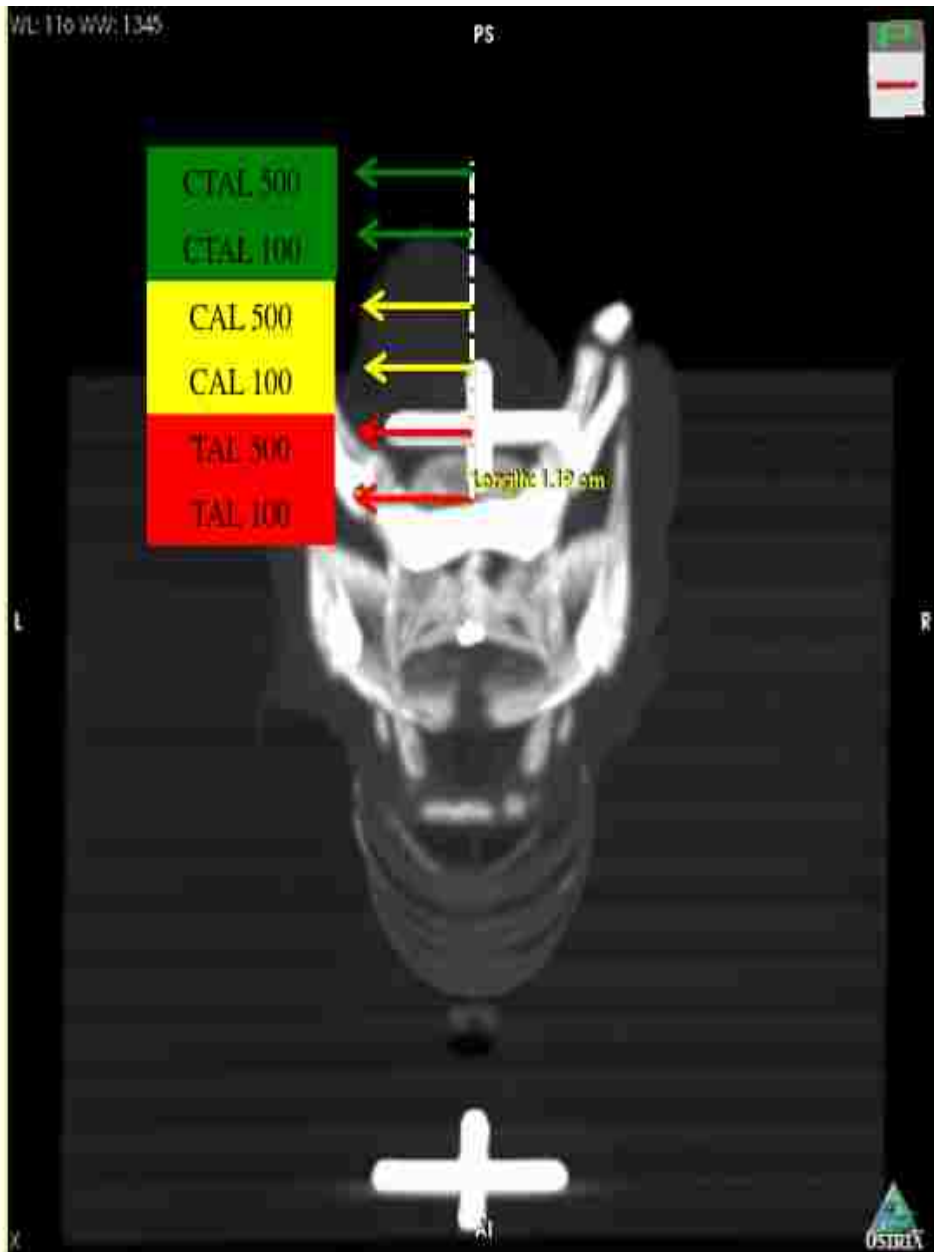
Fig 15. fid horiz means, MIP reconstruction
This figure shows a representative MIP reconstruction view of a larynx. The lines shown for each surgical procedure are scaled to represent the mean horizontal motion of the arytenoid fiducial marker (fid horiz). No significant differences are seen between procedures.

4.4 Vertical fiducial marker displacement relative to the laryngeal axis (fid vert)

No significant trends or associations are seen when comparing suture tension or technique. Suture tension and technique within arytenoid lateralization have no effect on fid vert. No comparison can be made with presurgical values since no fiducial marker may be placed prior to surgery. The mean fid vert data is illustrated in table 4 and fig 16 is a box and whisker plot normalized to dissected data. Table 5 shows the p values for pairwise comparisons between procedures. No significant differences are seen. Figure 17 shows a representative MIP reconstruction view of a larynx. The lines shown for each surgical procedure are scaled to represent the mean vertical displacement of the arytenoid fiducial marker (fid vert).
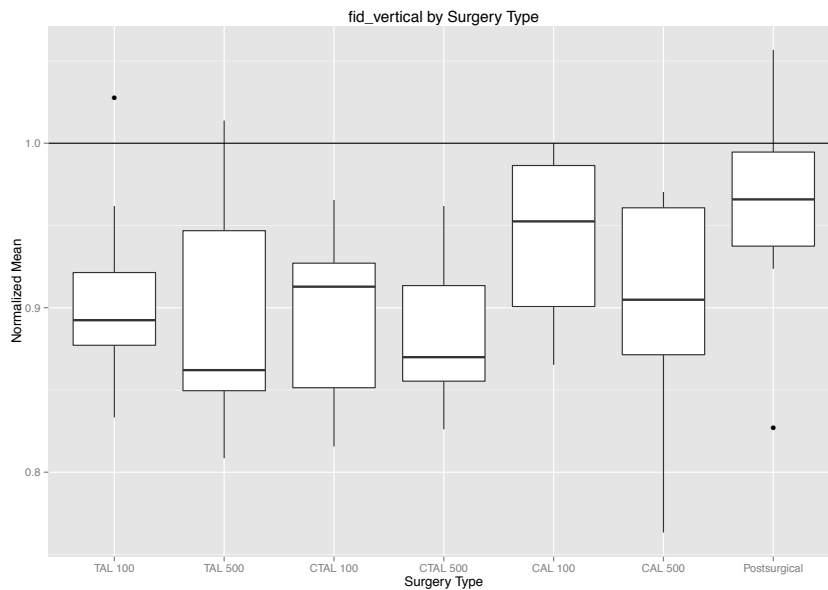
Fig 16. fid vert, box and whiskers plot

This figure shows the box and whiskers plot for the normalized means of vertical motion of the arytenoid fiducial marker (Fid Vert) by surgical technique. Increasing tension shows a trend for reducing mean fid vert. No significant differences are noted between different surgery types.

Fig 17. **fid vert means, MIP reconstruction**
This figure shows a representative MIP reconstruction view of a larynx. The lines shown for each surgical procedure are scaled to represent the mean vertical motion of the arytenoid fiducial marker (fid vert). No significant differences are seen between procedures.

Table 5. fid vert and fid horiz, pairwise comparisons
This table shows the p values for pairwise comparison between arytenoid lateralization surgeries of different types and suture tension. Values for Horizontal motion of the arytenoid fiducial marker (Fid Horizontal) and vertical (Fid Vertical) motion of the arytenoid fiducial marker are shown concurrently.

Surgical Intervention	TAL100	TAL500	CAL100	CAL 500	CTAL 100	CTAL 500	
TAL100		1.000	0.954	1.000	1.000	0.993	Fid vert
TAL500	1.000		0.767	1.000	1.000	1.000	
CAL100	0.954	0.728		0.565	0.781	0.565	
CAL 500	1.000	0.973	1.000		1.000	1.000	
CTAL 100	1.000	1.000	0.650	0.949		1.000	
CTAL 500	0.993	0.992	0.244	0.621	0.997		
	Fid horiz						

4.5 Caudal arytenoid displacement (CauAM)

CAL 500 (the high tension CAL suture) shows the lowest mean CauAM. Minor reduction in mean displacement is seen with increase in suture tension for a set technique but no significant trends or associations are seen between CauAM measurements. Suture tension and technique within arytenoid lateralization have no significant effect on caudal displacement of the arytenoid. No comparison can be made with presurgical values since no fiducial marker may be placed prior to surgery. The mean and standard deviation are shown in table 6 and fig 18 shows a box and whiskers plot. Table 7 shows the pairwise comparison for CauAM by surgery types. Figure 19 shows a representative MIP reconstruction view of a larynx. The lines shown for each surgical procedure are scaled to represent the caudal displacement of the arytenoid fiducial marker along the conserved laryngeal axis (CauAM).

Table 6. CauAM mean +-SD
This table shows the mean and standard deviation for caudal motion of the arytenoid fiducial marker (CauAM) measured on CT 3D reconstruction of the larynx.

Surgical Intervention	CauAM (mm)	
	Means	SD
Dissected	21.51	1.66
TAL100	19.68	1.63
TAL500	19.68	1.69
CAL100	19.98	1.64
CAL 500	18.81	2.08
CTAL 100	19.51	2.22
CTAL 500	19.58	1.66
Postsurgical	21.16	2.06
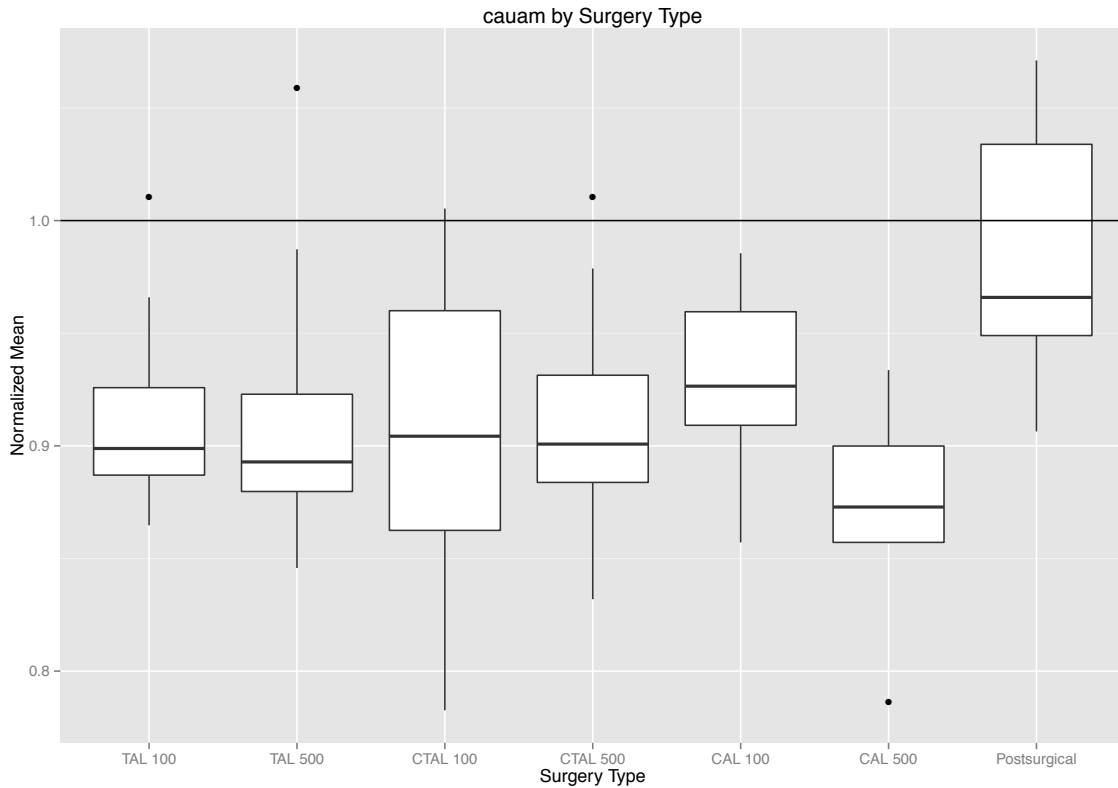
Fig 18. CauAM, box and whiskers plot
This figure shows the box and whiskers plot for the normalized means of caudal motion of the arytenoid fiducial marker (CauAM) by surgical technique. No significant differences are noted between different surgery types.
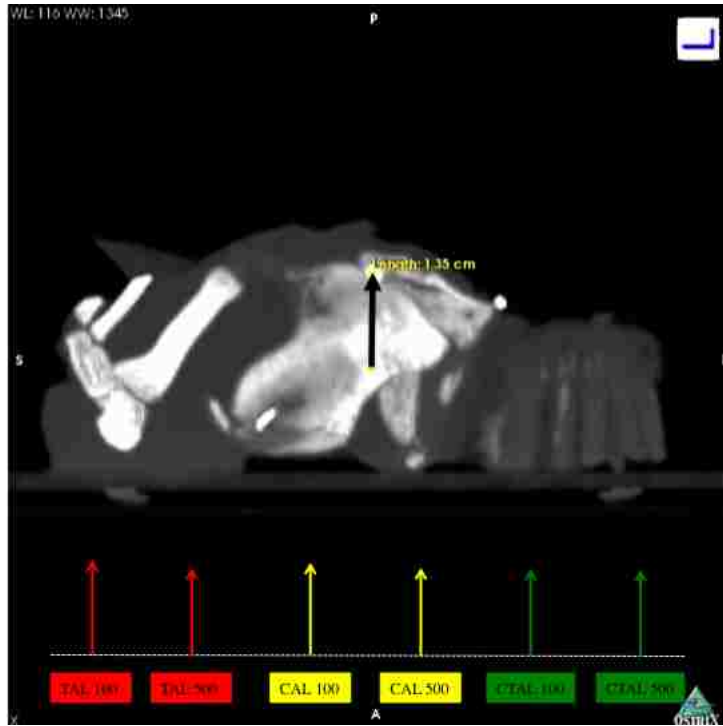

Table 7. CauAM, pairwise comparisons
This table shows the p values for pairwise comparison between arytenoid lateralization surgeries of different types and suture tension, for caudal motion of the arytenoid fiducial marker (CauAM).

Surgical Intervention	TAL100	TAL500	CAL100	CAL 500	CTAL 100
TAL500	1.000				
CAL100	1.000	1.000			
CAL 500	0.761	0.734	0.441		
CTAL 100	1.000	1.000	0.992	0.905	
CTAL 500	1.000	1.000	0.998	0.843	1.000
Caudal Arytenoid Motion, CauAM					

Fig 19. CauAM means, MIP reconstruction
This figure shows a representative MIP reconstruction view of a larynx. The lines shown for each surgical procedure are scaled to represent the caudal displacement of the arytenoid fiducial marker along the conserved laryngeal axis (CauAM). No significant differences are seen between procedures.

4.6 Inner dorsal arytenoid process distance (IAD)

Table 8 and fig 20 show that the distance between the right and left arytenoid cartilages does not change significantly between presurgical values and any technique, or between any techniques. Table 10 shows the p values for comparison between techniques and tensions. Figure 20 shows the box and whiskers plot for the normalized data of the interarytenoid distance. Figure 21 shows a representative rostral 3D reconstruction view of a larynx. The lines shown for each surgical procedure are scaled to represent the interarytenoid distance. No significant differences are seen between procedures.

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Table 8. IAD, mean +-SD, pairwise comparisons
This table shows the mean and standard deviation for the interarytenoid distance (IAD) measured on the rostral view of the 3D reconstruction of the larynx. p values shown are for pairwise comparison to presurgical values (p<0.05 is taken as significant).

Surgical Intervention	IAD (mm)		p value, pairwise comparison to presurgical
	Mean	SD	
Presurgical	6.01	1.27	
Dissected	6.16	1.07	
TAL100	7.36	3.11	1.00
TAL500	6.18	1.74	0.74
CAL100	6.54	1.50	1.00
CAL 500	6.73	1.52	0.98
CTAL 100	6.44	1.48	1.00
CTAL 500	6.75	1.78	0.98
Postsurgical	5.56	0.96	0.99
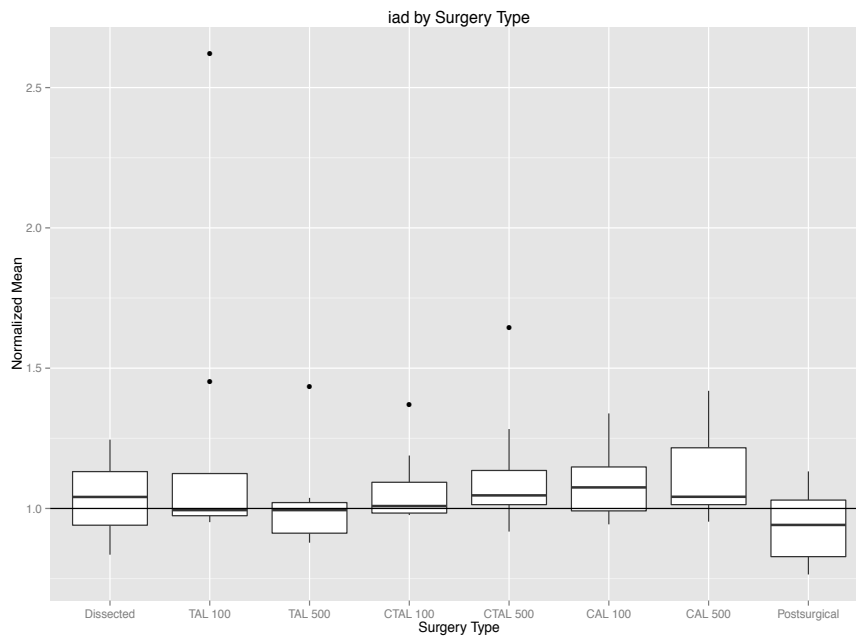
Fig 20. IAD, box and whiskers plot

This figure shows the box and whiskers plot for the normalized data of the interarytenoid distance. No significant differences are seen.
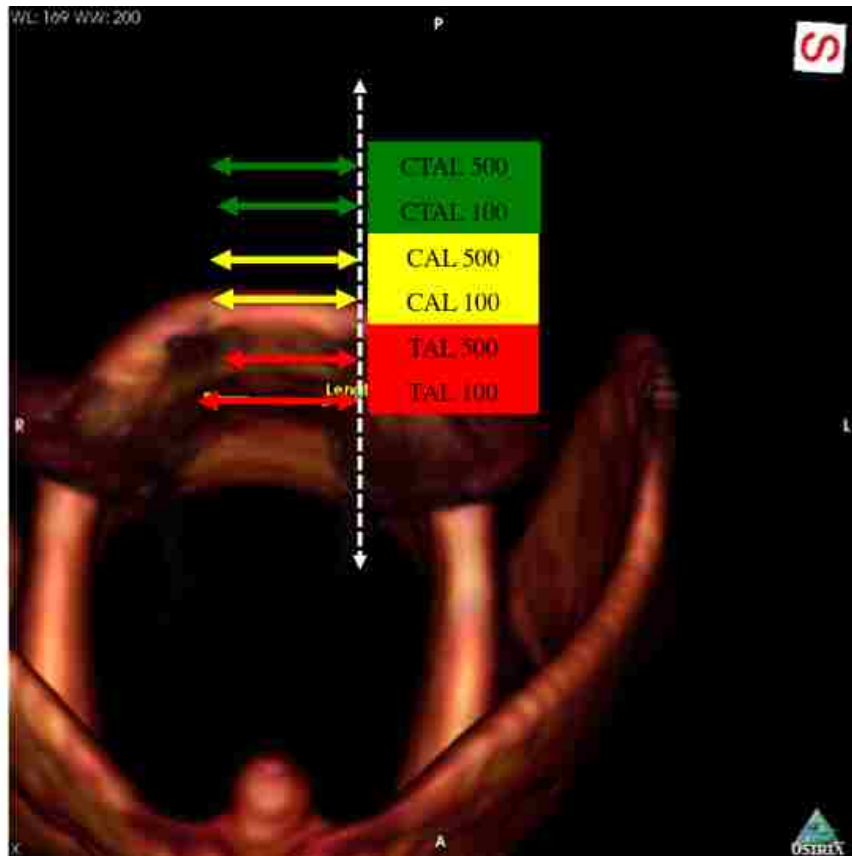
Fig 21. IAD, MIP reconstruction
This figure shows a representative rostral 3D reconstruction view of a larynx. The lines shown for each surgical procedure are scaled to represent the interarytenoid distance. No significant differences are seen between procedures.

4.7 Arytenoid to external thyroid wing distance (ATW)

Significant differences were seen between CAL 100 and CTAL 500 ($p < 0.005$) and between CAL 100 and TAL 500 ($p < 0.012$) in the distance between arytenoid cartilage and thyroid wing measured from the rostral view of the larynx.. Table 9 shows the mean +-SD data for ATW. There is a tendency for CAL procedures to have higher ATW distances than TAL and CTAL procedures. Suture tension reduces ATW for all

techniques but this is not significant. A comparison of all other arytenoid lateralization techniques was unable able to show a significant difference.

Fig 22 shows the box and whiskers plot with significances noted. Table 10 shows the p values for the pairwise comparison of techniques. Figure 23 shows a representative rostral 3D reconstruction view of a larynx with scaled data plotted to show the mean ATW for each procedure. Fig 24 shows the best illustration of the data by plotting the mean ATW distance between the CAL 100, TAL 500 and CTAL 500 on an example rostral view of a larynx. Table 9 shows the p values for comparison between techniques. The Shapiro-Wilk normality test in fig 25 suggests that ATW data is normally distributed validating the use of parametric statistics. Data to ensure that order of data and previous surgeries are not significant is shown in fig 27.

**Table 9. ATW, mean +-SD, pairwise comparisons** This table shows the mean and standard deviation for the arytenoid to thyroid wing distance (ATW) measured on the rostral view of the 3D reconstruction of the larynx. p values shown are for pairwise comparison to presurgical values (p<0.05 is taken as significant).

Surgical Intervention	ATW (mm)		p value, pairwise comparison to presurgical
	Mean	**SD**	
Presurgical	15.26	1.33	
Dissected	16.84	1.77	
TAL100	15.40	1.12	1.00
TAL500	14.84	0.89	0.99
CAL100	16.54	1.62	1.00
CAL 500	15.94	1.66	0.99
CTAL 100	15.30	1.13	0.51
CTAL 500	14.73	1.27	0.97
Postsurgical	16.91	1.63	0.18

Fig 22. ATW, box and whiskers plot
This figure shows the box and whiskers plot for the normalized means of the arytenoid to thyroid wind distance (ATW). Significant differences were seen between CAL 100 and CTAL 500 (p<0.005) and between CAL 100 and TAL 500 (p<0.012)
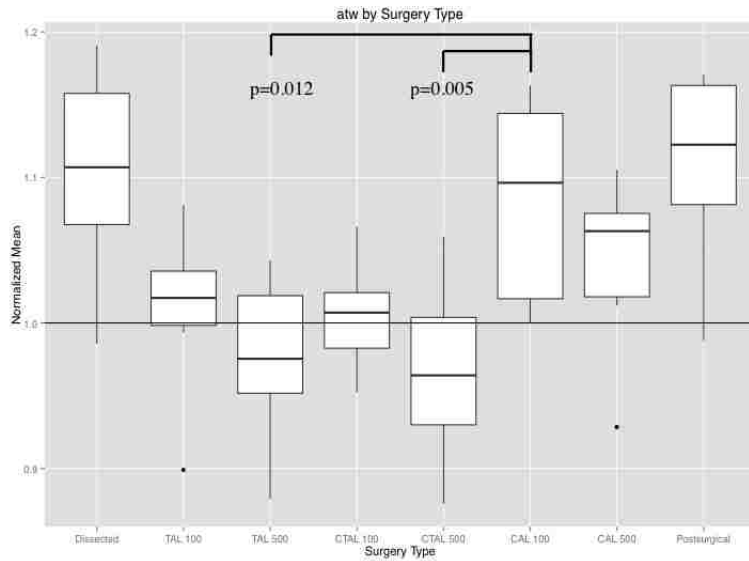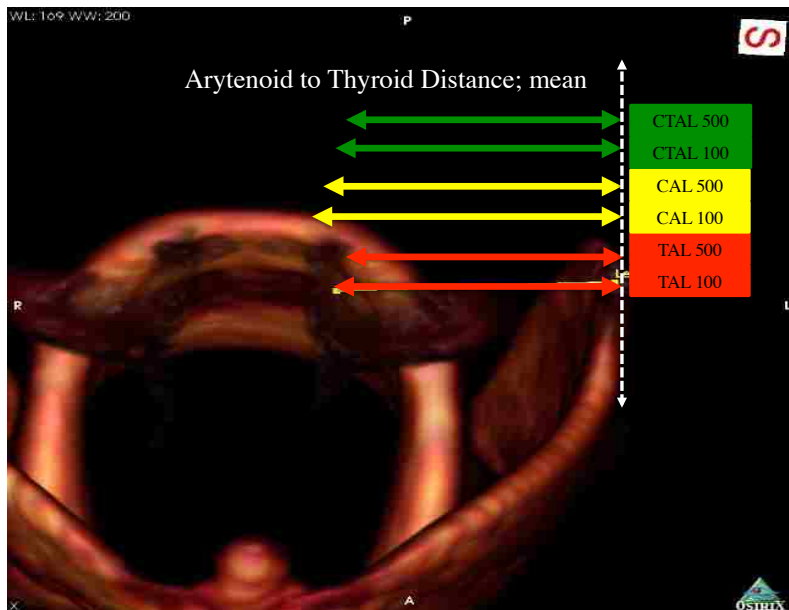

Fig 23. ATW, MIP reconstruction, all surgeries
This figure shows a representative rostral 3D reconstruction view of a larynx. The lines shown for each surgical procedure are scaled to represent the distance between the

arytenoid and the thyroid wing (ATW). Significant differences include CAL 100 and TAL 500 (p=0.012) and between CAL 100 and CTAL 500 (p=0.005) which are illustrated in figure 24.
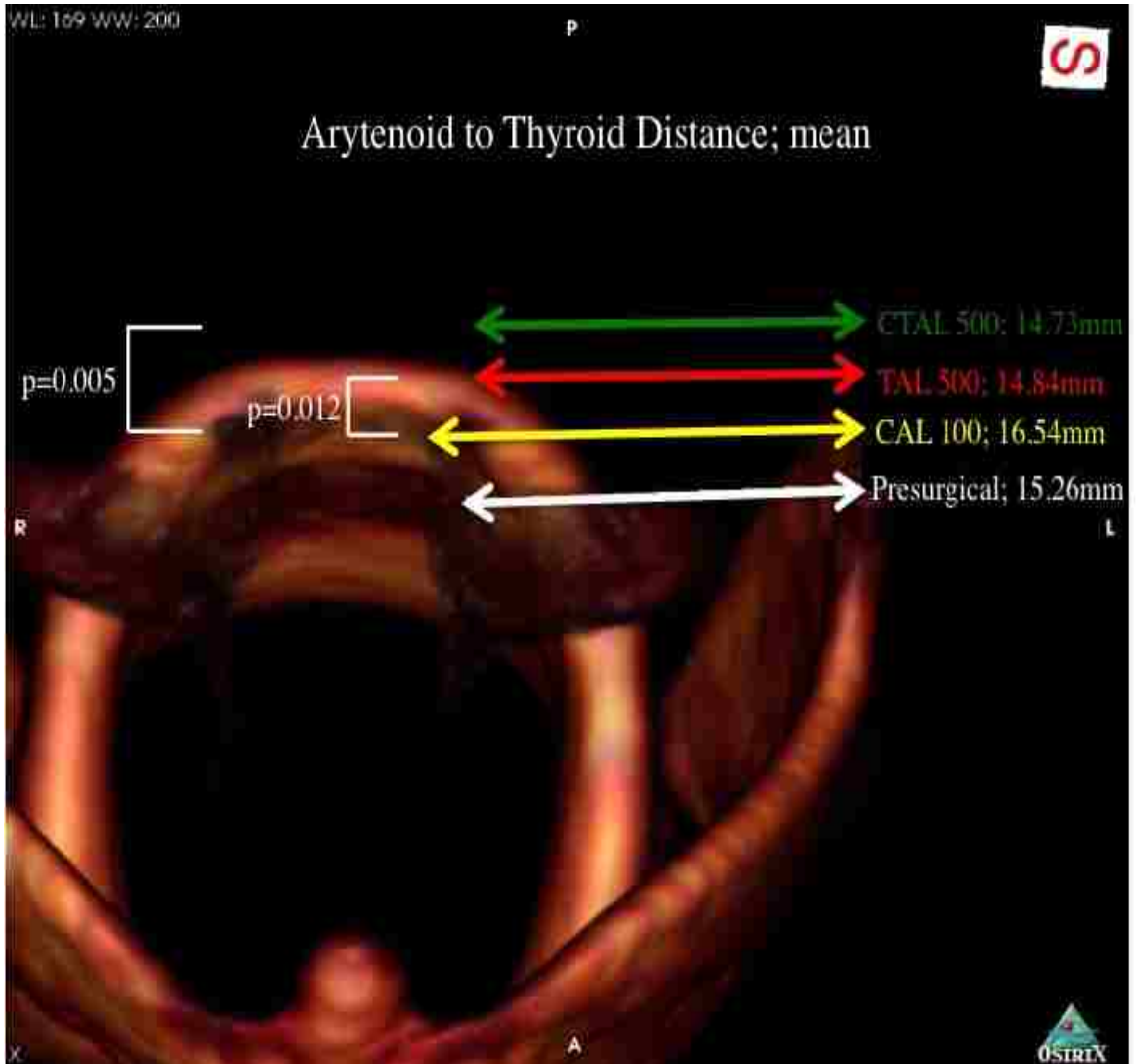
Fig 24. ATW, MIP reconstruction, significant differences
This figure shows the rostral view whereby ATW measurements are made. This is a presurgical larynx (no fiducial marker placed) and the white line (presurgical) shows the measured distance. The other arrowed lines correspond to the means ATW of CAL 100 (yellow), TAL 500 (red) and CTAL 500 (green) to illustrate the difference in mean distance for these values. Significant differences are shown between CAL 100 and TAL 500 (p=0.012) and between CAL 100 and CTAL 500 (p=0.005).
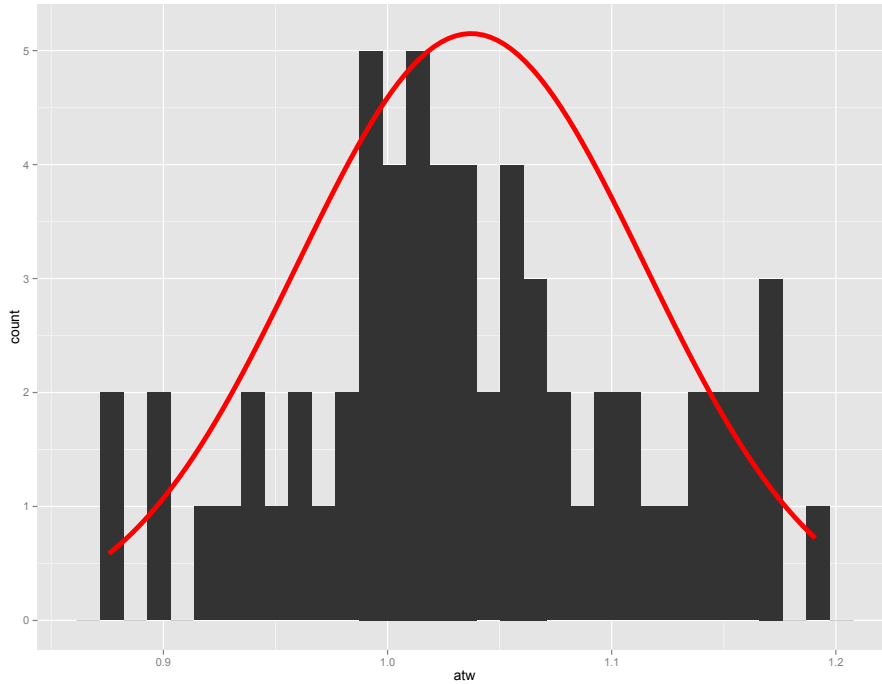
Fig 25. ATW, departure from normality
Shapiro-Wilk test for departure from normality for arytenoid to thyroid wing distance (ATW), for all larynges. p-value = 0.3199 suggesting normal distribution.

Table 10. ATW and IAD, pairwise comparisons
This table shows the p values for the pairwise comparison between surgical techniques for the arytenoid to thyroid wing distance (ATW) and for the interarytenoid distance (IAD) measured on the rostral view of the 3D reconstruction of the larynx. p<0.05 is taken as significant and highlighted in red.

Surgical Intervention	TAL100	TAL500	CAL100	CAL 500	CTAL 100	CTAL 500	
TAL100		0.662	0.922	0.981	0.877	0.984	
TAL500	0.926		1.000	0.992	1.000	0.990	
CAL100	0.237	0.012		1.000	1.000	1.000	IAD
CAL 500	0.956	0.311	0.871		1.000	1.000	
CTAL 100	1.000	0.978	0.144	0.879		1.000	
CTAL 500	0.804	1.000	0.005	0.181	0.912		
	ATW						

4.8 Conserved laryngeal axis length (CLA)

Conserved laryngeal axis length was not significantly different between any interventions on the same larynx, suggesting no relative movement between the thyroid and cricoid cartilages. Table 11 and fig 26 show small variations in mean and median values and table 12 illustrates the p values from the pairwise comparisons of all procedures. No significant differences are noted.

Table 11. CLA, mean +-SD
This table shows the mean and standard deviation for the conserved laryngeal axis (CLA).

Surgical Intervention	CLA (mm)	
	Mean	**SD**
Presurgical	NA	NA
Dissected	39.82	2.61
TAL100	39.41	2.83
TAL500	39.49	2.76
CAL100	39.48	2.67
CAL 500	39.21	3.03
CTAL 100	39.46	3.12
CTAL 500	39.43	3.14
Postsurgical	39.35	3.12

4.9 Dissected vs. postsurgical

A comparison of the dissected larynx (whereby sutures have been placed but not tightened) and the post surgical larynx (whereby sutures have been repeatedly tightened and loosened for each experiment and then left loose) was performed to identify any changes in measured parameters that might be caused by repeated tensioning. Table 13
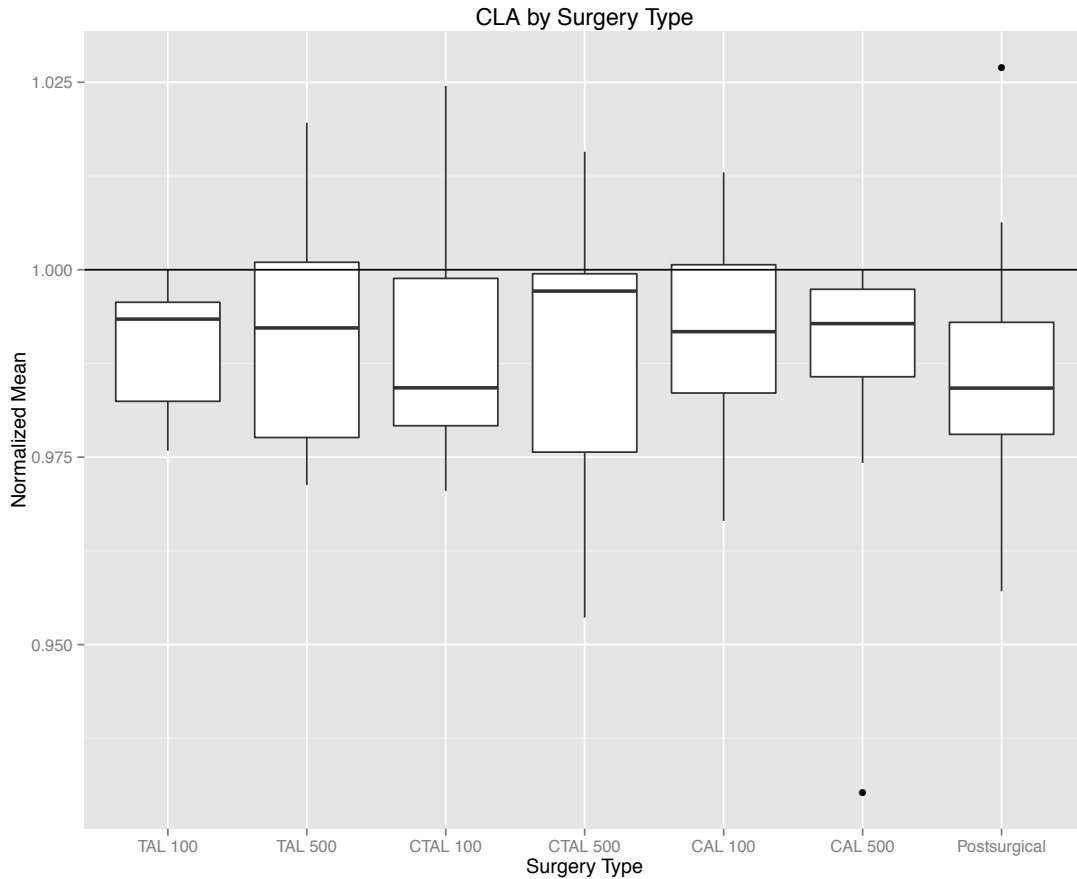
Fig 26. CLA, box and whiskers plot
This figure shows the box and whiskers plot for the normalized data of the conserved laryngeal axis (CLA). No significant differences are seen.

shows the means of such data and the p values from pairwise comparisons. No significant differences are seen in any of the measured parameters. Figure 27 shows a Kernel distribution of bootstrapped means specifically for ATW since significant differences were seen. One hundred bootstrap samples were taken of these values and the densities are shown above. Visually the densities appear similar. This gives us confidence that larynges are not materially affected by sequential surgeries at the level of measurement we were able to carry out.

Table 12. CLA, pairwise comparisons
This table shows the p values for the pairwise comparison between surgical techniques for the Conserved Laryngeal Axis (CLA). p<0.05 is taken as significant.

Surgical Intervention	TAL100	TAL500	CAL100	CAL 500	CTAL 100
TAL100					
TAL500	1.000				
CAL100	1.000	1.000			
CAL 500	0.998	0.987	0.990		
CTAL 100	1.000	1.000	1.000	0.996	
CTAL 500	1.000	1.000	1.000	0.999	1.000
Conserved laryngeal axis, CLA					

Table 13. Dissected vs. postsurgical, mean +-SD, pairwise comparisons
This table shows the means and standard deviation for all variables between the dissected and the postsurgical larynx. p values are given for the pairwise comparison dissected and the postsurgical larynx (p<0.05 is taken as significant). This is a comparison of larynges after dissection and placement of sutures (dissected) and at the end of the procedure when the sutures have been sequentially tied and loosened (postsurgical). No significant changes are seen in measured parameters are seen by sequential suture tightening.

Surgical Intervention	Means	Cranial view ATW (mm)	IAD (mm)	Volume measurement Laryngeal Volume (cm³)	MIP Central CSA (cm²)	3D reconstruction Cau AM (mm)	Fid Horiz (mm)	Fid Vert (mm)	CLA (mm)
Dissected		16.84	6.16	7.24	0.80	21.51	12.55	14.33	39.82
Postsurgical		16.91	5.56	7.15	0.68	21.16	13.06	13.78	39.35
P value dissected vs. post surgical		1.000	0.994	1.000	0.999	0.999	0.896	0.986	0.817
	SD	ATW (mm)	IAD (mm)	Laryngeal Volume (cm³)	Central CSA (cm²)	Cau AM (mm)	Fid Horiz (mm)	Fid Vert (mm)	CLA (mm)
Dissected		1.77	1.07	1.84	0.33	1.66	1.17	0.86	2.61
Postsurgical		1.63	0.96	1.63	0.15	2.06	2.04	1.14	3.12

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Fig 27. Kernel distribution of bootstrapped means; a comparison of dissected and postsurgical

This figure 27 shows a Kernel distribution of bootstrapped means specifically for ATW. One hundred bootstrap samples were taken of these ATW values for dissected and postsurgical data and the densities are shown above. Visually the densities appear similar. This gives confidence that larynges are not materially affected by sequential surgeries at the level of measurement we were able to carry out.
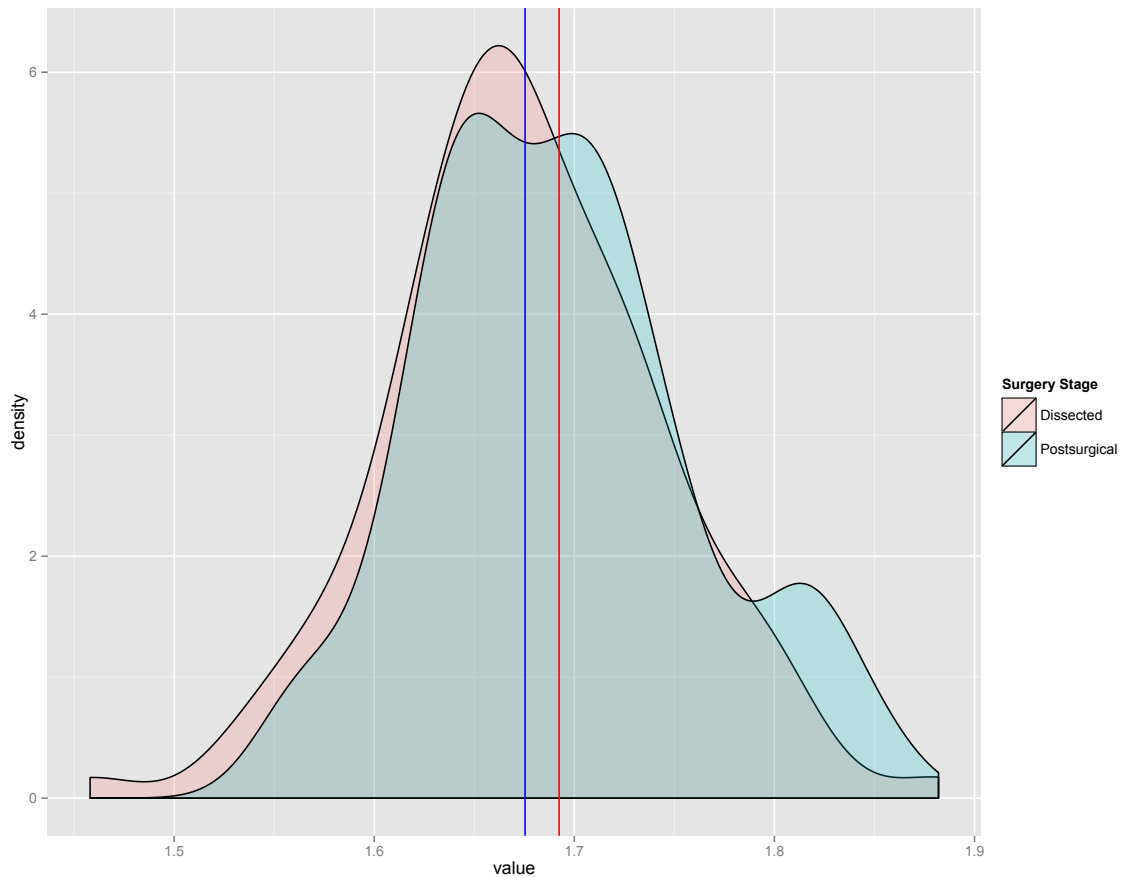
4.10 Arytenoid displacement

Figures 28 and 29 compile the best visual appraisal of the effect of technique and

tension on measured parameters. ATW and fid horizon represent lateralization of the

larynx (ATW decreases with increasing lateralization and fid horiz increases with

increasing lateralization). Fid vertical is the only measure of vertical displacement and

CauAM represents caudodorsal displacement parallel to the conserved laryngeal axis.

For a set tension of 100g, there is a tendency for CAL procedures to cause less vertical

and more caudal displacement than the TAL, which produces a greater lateralization.

This is illustrated in fig 28. At a tension of 500g, there is a similar tendency for CAL

procedures to cause less lateralization than TAL procedures, shown in fig 29.
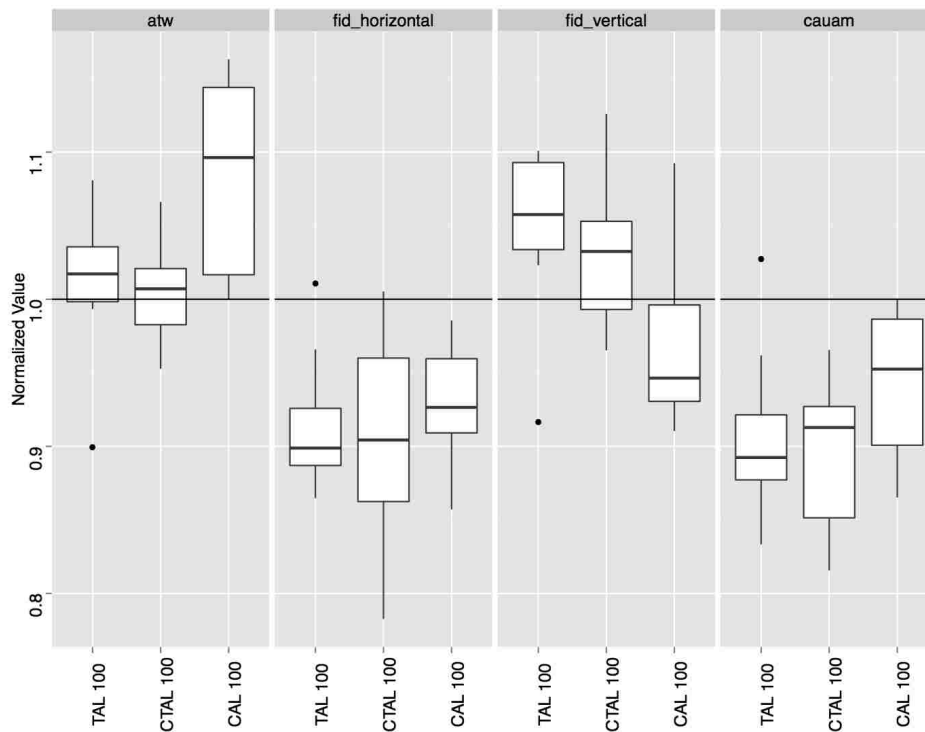
Fig 28; Combined box and whiskers plot for arytenoid displacement in three planes, at 100g suture tension

This box and whiskers plot displays the combined affect on the lateralization (ATW (small values indicate more lateralization) and fid horiz (larger values represent more lateralization)), vertical motion (fid vert) and caudodorsal motion (CauAM) or the arytenoid due to different UAL techniques. For a set tension of 100g, there is a tendency for CAL procedures to cause less vertical and more caudal displacement than the TAL, which produces a greater lateralization.
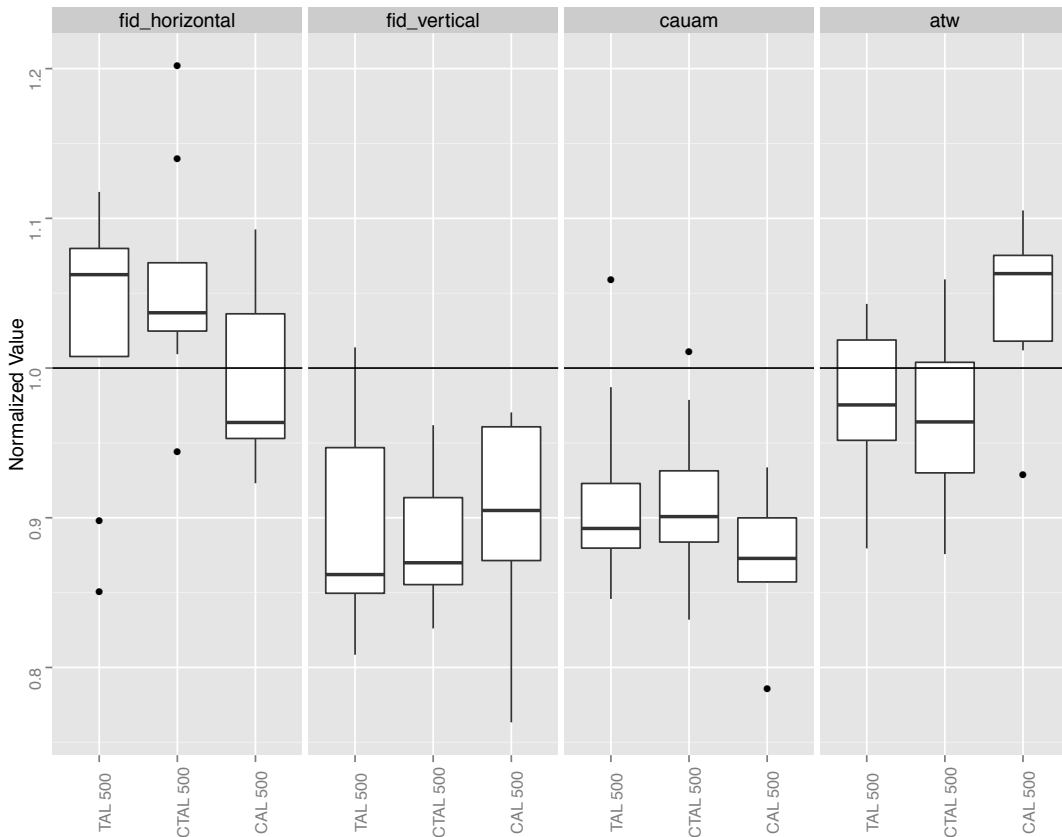
Fig 29. Combined box and whiskers plot for arytenoid displacement in three planes, at 500g suture tension

This box and whiskers plot displays the combined effect on the lateralization (ATW (smaller values indicate more lateralization) and fid horiz (larger values represent more lateralization)), vertical motion (fid vert) and caudodorsal motion (CauAM) or the arytenoid due to different UAL techniques. For a set tension of 500g, there is a tendency for CAL procedures to cause less lateralization than the TAL and CTAL procedures.

5. DISCUSSION

All CT scans were able to distinguish the arytenoid cartilage from the thyroid and cricoid cartilage. The method described was easy to perform and repeatable.

ATW and lateralization

This study found significant changes in ATW when a high tension TAL suture was used as part of the surgical treatment (TAL 500 and CTAL 500) compared to a low tension CAL. TAL is postulated to move the arytenoid more laterally[3]. The significant difference found in this study may correspond to excessive lateralization and may correlate to a similar mismatch between the RG and the epiglottis as noted by Bureau[31]. In that study increased tension and further dissection in a CAL may predispose to aspiration pneumonia by creating a mismatch between the RG and epiglottis and allowing an 'aperture' between the two for fluid or food to be aspirated[31]. A lower closed glottal resistance, as an assessment for the seal created by the glottis and epiglottis, was found by Greenberg at al. for the same subjectively measured increase in tension for CAL procedures[5].

No significant changes were seen on any measures of the arytenoid fiducial marker motion when comparing arytenoid lateralization techniques controlled for tension. This suggests that lateralization techniques achieve very similar effects on arytenoid motion except for ATW. The use of a high tension TAL suture caused a significant effect on lateralization without significantly affecting other measured parameters. These

techniques were compared by the same surgeon in a previous study[10], and CAL and CTAL procedures were shown to reduce airway pressure compared to TAL procedures. No significant differences were seen between CAL and CTAL procedures. Reduced airway pressure is expected to improve clinical outcome, unless it leads to excessive lateralization and an increased predisposition to aspiration pneumonia. This data suggests that CAL procedures are able to provide significant reductions in airway pressure, without excessive lateralization measured by a significant reduction in ATW.

Comparisons between surgical procedures performed by different surgeons are commonly made when evaluating literature and the translation of surgical information relies on the assumption that inter-operator variability between surgeons is low, however caution should be taken in correlating the results of airway pressure experiments with this CT study. Intraoperator differences and effects are unknown for repeated laryngeal surgeries.

CTAL and TAL at 500g of tension achieve significant reductions in ATW compared to CAL 100g but not fid horiz. Both of these measures were designed to assess lateral motion of the arytenoid. Fid horiz shows a similar trend without showing statistical significance; this can be explained by the lower effect size of fid horiz than ATW, shown in figure 31.

The effects of Arytenoid lateralization technique alone

This study examined the effects of TAL, CAL and CTAL procedures on the 3D structure of the larynx and displacement of the arytenoid cartilage, which can be summarized in figures 28 and 29. Subjective observation of the laryngeal lumen shows deformation at the rima glottis in a dorsolateral direction, which is most prominent for high-tension TAL and CTAL procedures. On sagittal observation, minor arytenoid translation is observed rather than tilting of the arytenoid cartilage. Data suggests that although minor changes are present for different surgical techniques, measures of laryngeal volume and arytenoid position are not significantly different between TAL, CAL and CTAL procedures when tension and other aspects of technique remain the same. No study has been able to show a significant difference in clinical outcome between these techniques, however some objective changes have been seen by the measurement of RGA and airway pressure. CAL has been shown to increase RGA compared to TAL *in vivo*[8] and in cadaver studies[9] and reduced airway pressure compared to TAL in cadaver studies[10].

The effects of suture tension alone

The effects of suture tension (i.e. comparisons of TAL 100 vs. TAL 500, CAL 100 vs. CAL 500 and CTAL 100 vs. CTAL 500) revealed no significant differences, although examination of the data suggests non-significant trends in increasing fid horiz (fig 14) and reductions in fid vert (fig 16) for all techniques. Subjective observation of the laryngeal lumen shows deformation at the rima glottis in a dorsolateral direction, which is most prominent for high-tension procedures. On sagittal observation, minor arytenoid

translation is observed rather than tilting of the arytenoid cartilage. In previous cadaver investigations of unilateral CAL, lower suture tension has been shown to cause a smaller increase in RGA[31] and further dissection with increased suture tension has been found to have no effect on laryngeal resistance[5]. A previous study from this author which performed the same UAL procedures while measuring airway pressure, found that increased suture tension led to significant changes is airway pressures were seen in TAL at air flow rates of 30L/min and CAL at air flow rates of 45-120L/min, with 30 and 60L/min being recorded in normal dogs and those with laryngeal paralysis respectively.[10] Suture tension employed in clinical cases of UAL has not been compared to clinical outcome. The present study showed that increasing tension alone has no significant effect on measured CT parameters of laryngeal volume and arytenoid position. The CAL suture is postulated to recreate the function of the cricoarytenoideus dorsalis muscle[3], abducting the arytenoid and drawing it in a caudal direction, along the laryngeal axis. Type II error cannot be ruled out and CT may have been unable to detect a statistically significant difference.

Volume

No significant changes were found in the volume of the larynx, suggesting that lateralization technique and tension have no effect on volume and that volume would not correlate with airflow. The internal volume and internal surface of a tube are expected to have an effect on airflow through a tube due to generation of turbulent flow which exists at higher pressure than laminar flow[18]. The idea of the larynx being a single aperture at the end of a tube may be an oversimplification. Additional less

significant points of narrowing or airway turbulence, caudal to the RG could have an effect on airflow through the upper airway. The ventricular folds and vocal folds are implemented as significant source of airway resistance in the computer simulated models of the human larynx and trachea[22]. This study showed that UAL technique and tension has no significant effect on laryngeal volume and a tendency to reduce volume rather than increase volume. Increased volume would be an expected result to improve airflow through the larynx, although if the RG is the most significant source of narrowing volume may have limited clinical significance. The limitations of volume measurement on CT are that measurement may only occur when the airway is surrounded 360° by tissue, therefore it is measured within the fixed overlapping structure of the cricoid and thyroid cartilage. Because of the caudal slope of the RG relative to the long axis of the larynx measured on CT, this omits the volume of air between the vocal folds, which might be expected to change with RG size. This volume of air is illustrated in figure 30.

Given that the cricoid and thyroid cartilages are two semi rigid structures with an intact adjoining cricothyroid ligament, it is expected that UAL would have no significant effect on laryngeal volume measured in this study.

Cross sectional area at the central to rostral section of the larynx (cCSA) shows an interesting pattern of results. Increase in tension increases cCSA and CAL/CTAL procedures show increases in cCSA compared to TAL procedures, results shared in
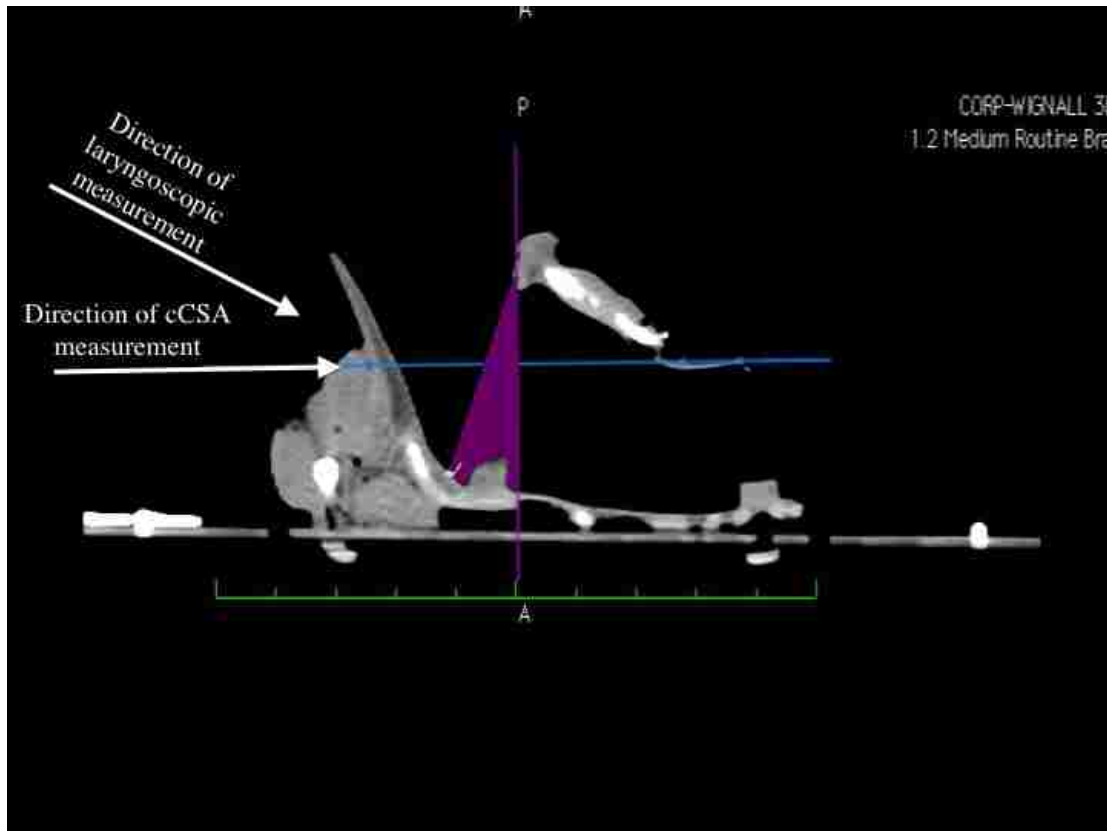
Fig 30. Cranial unmeasured laryngeal volume and the comparison of cCSA and RGA measurement techniques

As is fig 3a, this shows a sagittal projection of the larynx taken from multiplanar reconstruction. The area highlighted in purple is the cranial volume of the larynx, which could not be included in the measurement of laryngeal volume. The white arrows show the direction from which cCSA is measured in the axial slice on CT and the typical direction of laryngoscopic measurement of RGA, which is typically performed with cranial epiglottic traction.

measurements of RGA and the inverse of airway pressure data in this authors previous study. Statistical analysis shows that the differences in mean are unlikely to be significant. Due to the variation between laryngeal and standard deviation of +- 0.3 cm^3 for the presurgical larynges, a difference in cCSA of approximately 0.2cm^3 would be required to give this test statistical significance. Although cCSA does incorporate the dorsal and ventral limits of the RGA as measured by laryngoscopy, it measures from an

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alternate angle, measuring the end on view of a tilted RG, which would expect to be lower. This is illustrated in figure 30.

The interarytenoid distance is not significantly affected by surgical technique or tension. This is an expected finding given that this surgical technique maintains the interarytenoid band, a cartilaginous attachment between the left and right arytenoid cartilages. It has been hypothesized that interarytenoid band transection allows dorso-lateral arytenoid movement to cause greater increase in RGA[3]. One author[9] found that sectioning the IA caused significant reduction in the dorsoventral height of the *rima glottidis* but no significant increase in RGA. A recent study showed that sectioning of the interarytenoid band had no effect on airway pressure, but for a low tension TAL procedure, it did allow an increase in RGA[10]. Given this information, repeating the present study with IA band sectioning might allow greater movement of the arytenoid and more detectable differences in technique or tension. A previous study found that IA sectioning can increase the RGA achieved by a TAL procedure without affecting airway pressure[10].

It is important to note that statistical significance does not necessarily equal clinical significance in this study, and this study is not designed to evaluate clinical effect. Airflow through any tube is represented by $R=8nl/\pi r^4$ where R= resistance, n= gas viscosity, l= length of tube and r = radius of tube[18], whereby small changes in radius can greatly affect airway resistance and pressure.

Interpretation of CT measurements and potential use in prospective analysis

In a single clinical patient both visual assessment and Osirix based measurements could be used to objectively assess the measurable effects of surgery. Example CT images of the mean effects of each procedure are available for each measured parameter (Figures 15 (Fid horiz), 17 (Fid vert), 19 (CauAM) and figure 21 (IAD)) and show minimal visible movement of the arytenoid cartilage and fiducial markers, which would be unlikely to allow an observer to distinguish between techniques, suggesting either that there is no difference in the effects of techniques and tensions or a lack of sensitivity for these CT measures. The significant differences seen for ATW in figures 23 and 24 may be more likely to yield a result from an observer, however a prospective analysis would rely on the measurement of ATW on a 3D CT reconstruction to objectively grade the level of lateralization achieved by UAL and compare to clinical outcome.

Limitations

Cadaver studies for regional CT have the benefit of removing extraneous tissue that could contribute to artifact but also it is unknown whether loss of supporting tissue could affect the structure of the larynx. A small number of dogs were used in this study however using a single surgeon with repeatable technique and consistent placement of the suture [45] should limit variation between the surgical dose and allow the larynges to act as their own control. As with previous studies[8,10], all CTAL procedures were performed by tightening the CAL suture prior to the TAL suture and the effect of reversing this is unknown and has not been previously studied.

It is important to remember that this study does not take into account the effect of dynamic motion within the larynx or the effects of inspiration and expiration on measured CT parameters. The excised larynx may deform at high airway pressures which has been documented in a previous study by this author [10]. This deformation would be expected to affect CT measurements such as those performed here. The application of a set suture tension may be of questionable clinical significance, as no information exists as to the inter-operator variation in suture tying. In this study objective tensioning is used to eliminate heterogeneity in surgical technique and to allow a basis for prospective study. This study was not designed to assess the clinical significance of different techniques of arytenoid lateralization. The aim of the study was to assess the effects of arytenoid lateralization on the measurable structure of the larynx, to allow inference about its value to clinical scenarios. No conclusions about clinical outcome can be made based on this data.

The amount of arytenoid motion is small, based on fid horiz and fid vertical data. As such, minor differences between procedures may not be detected or if detected may not be statistically significant. A much greater difference in arytenoid cartilage displacement would be expected by comparing the presurgical values to the arytenoid lateralization techniques. However, since the fiducial marker could not be placed prior to surgery, this investigation was unable to ascertain if significant arytenoid displacement occurs between the presurgical and larynx and the lateralized arytenoid. This could be achieved by the identification of a repeatable objective marker on the arytenoid cartilage itself. ATW was the only direct measure of arytenoid displacement

that could be compared before and after surgery, i.e. did not require a fiducial marker. This was the only variable significantly affected by technique and tensions. Placing any kind of fiducial marker, even an inert biomaterial could have the potential for post-operative migration, infection or fistulation, therefore any measurement used prospectively would ideally not involve placement of a fiducial marker. Dissected data was included to allow a source of normalization for this data, however an objective comparison of dissected data and any surgical technique would be clinically irrelevant.

Osirix measurement software was used during this study; no studies exist to assess the accuracy of such measurements on the larynx. One study based on drilling osseous tunnels into the porcine distal femur assessed the comparison between digital calipers and Osirix 3D reconstruction measurements. [46] The authors found the differences in measurements to be generally less than 0.1mm with a maximum difference of 0.3mm. This would suggest that the application of Osirix to this study could be achieved with a reasonable level of accuracy given the magnitude of measurements made during the study.

Rima Glottidis Area

RGA was not directly measured as in some previous studies, which precludes cross comparison of one documented effect of this study's lateralization procedures. Other studies have similarly omitted this measurement technique when evaluating cadaver laryngeal surgeries[5,39]. Virtual endoscopy in Osirix (Osirix foundation, Geneva) was unable to measure the equivalent value and unlike other studies, the epiglottis was not

retracted cranially (for laryngoscopic exam in live animals or for pinning in cadaver studies) which may limit the assessment of true RGA as previously measured. Although video laryngoscopy has been used for the assessment of the effect on anesthetic agent on the larynges of normal dogs[47], dogs with laryngeal paralysis[48] and normal cats[49], no information exists for references ranges for RGA measured by this method and no study has compared RGA measured on video laryngoscopy with standard laryngoscopic measurement of RGA or measurement in the cadaver larynx. cCSA incorporates the rostral 1.8mm segment of the larynx and includes the ventral and dorsal limits of the RG. cCSA can be compared to the corresponding RGA measured in a technique similar to laryngoscopic exam in this author's previous study (table 14). The same surgeon performed these procedures, but breed of dog is different. Data was normalized to minimize breed differences and to show that cCSA may be a different measurement compared to RGA. This may be due to the alternate angle of measurement, which is illustrated in figure 30.

As this study was aimed to recreate the natural position of the larynx of a sedated dog, it was important to leave the epiglottis in a neutral position. The effect of epiglottic retraction on RGA is currently unknown.

Power Study

Due to the low number of cadaver specimens in this study, the aim of this study was not to specifically evaluate the significance of each individual measure or it's power at distinguishing one surgery from another. The major aim of the study was to evaluate

Table 14. A comparison of the central to rostral cross sectional area of the larynx (cCSA) to *Rima Glottidis* area (RGA) directly measured with a cranially retracted epiglottis in Wignall et al. 2012

This table shows mean and normalized mean cCSA data compared to mean *Rima Glottidis* area (RGA) directly measured with a cranially retracted epiglottis in Wignall et al. 2012, for each surgical procedure. Procedures were performed by the same surgeon but on different larynges. Measurement techniques differ as shown in figure 30.

Surgical Intervention	MIP projection		Direct measurement (Wignall et al., 2012)
Means	**cCSA (cm²)**	**Normalized to presurgical**	**Normalized RGA**
Presurgical	0.81	100.00	100.00
TAL100	0.86	106.17	152.50
TAL500	0.87	106.91	161.20
CAL100	0.94	115.80	159.20
CAL 500	0.99	121.85	179.30
CTAL 100	0.92	113.33	164.20
CTAL 500	0.93	114.44	183.00

whether any of the CT measurements would be suitable for further investigation or prospective assessment in live patients. Due to the low power of some of the variables examined, shown in table 15, this study would not allow the generation and proving of the null hypotheses of non-significance for values of laryngeal volume, Fid horiz, Fid vert, CauAM and IAD.

A larger sample population or more homogenous sample might be required to prove or disprove the null hypothesis. Figure 31 shows the relationship between power and effect

Table 15. Power study for measured CT parameters
The table shows the statistical power for 8 dogs for each measured CT parameter.

Variable	Median Effect	Overall Mean	Power
CLA	0.009	0.991	0.050
Laryngeal volume	0.016	0.984	0.051
Fid horiz	0.036	1.020	0.053
CauAM	0.070	0.930	0.061
ATW	0.076	1.033	0.063
Fid vert	0.077	0.923	0.063
IAD	0.172	1.073	0.126
cCSA	0.182	1.106	0.137

size for each variable. Since this shows an exponential relationship, the recruitment of further numbers may increase statistical power considerably.
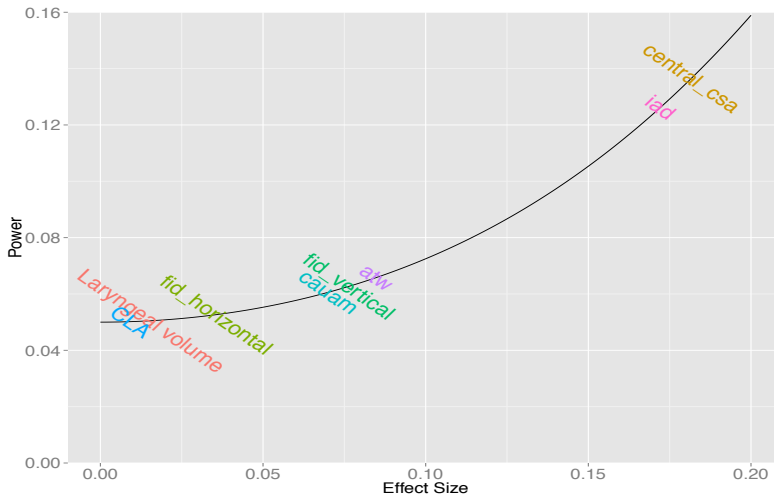
Fig 31. Power against effect size for measured parameters on CT
Figure 31 shows the relationship between power and effect size for each variable. Since this shows an exponential relationship, the recruitment of further numbers may increase statistical power considerably. In the number of patients likely included in a prospective veterinary study, significant differences for the majority of the measured parameters may be unlikely.

In the number of patients likely included in a prospective veterinary study, significant differences for the majority of the measured parameters would be unlikely. The fact that differences in ATW were found despite low power shows the strength of this difference, and that it would be a useable hypothesis for the typical low number of dogs involved in veterinary studies. This number of replicates has been used previously in similar studies but the effect size of variables in this study may be too small to allow the detection of significant differences between this many specimens [5,10,39,31].

Repeated measures

CT analysis of the larynx found no significant differences between the dissected larynx (fiducial markers and sutures placed, with no tension applied) and the postsurgical larynx (fiducial markers and sutures placed, with tension having been applied for six short periods of varied tension, then loosened), suggesting that no permanent deformation occurs by temporary suturing. Figure 27 shows a Kernel distribution of bootstrapped means specifically for ATW since significant differences were seen. One hundred samples were taken of these values and the densities are shown. Visually the densities appear similar. This gives us confidence that larynges are not materially affected by sequential surgeries at the level of measurement we were able to carry out. Mean CLA also remains within a small window of variability, particularly given the previously reported error level of Osirix measurements[46] suggesting that no significant movement occurs between the thyroid and cricoid cartilage.

6. CONCLUSIONS

Significant changes in the distance between the arytenoid cartilage and thyroid wing are seen between low-tension CAL procedures and high tension TAL and CTAL procedures.

The effects of all procedures and tensions on measured CT parameters are the same except for ATW. High tension TAL and CTAL procedures cause excessive lateralization of the arytenoid not found in low-tension CAL procedures. CAL may provide an optimum reduction in airway pressure as seen in previous studies[10], without excessive lateralization.

Prospective analysis could be performed on clinical laryngeal paralysis patients before and after UAL, with the hypothesis that dogs with a detectable increase in ATW may be predisposed to aspiration due to excessive lateralization.

The use of larynges for repeated measures has no visible effect on the anatomic structure as examined by this study's CT measures.

Greater numbers of canine larynges would be required to prove the remaining null hypotheses of this study however this would be unlikely to hold clinical significance given the typical low numbers of dogs is veterinary clinical studies.

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