Computed tomographic geometrical analysis of surgical treatments for equine recurrent laryngeal neuropathy

Michelle L. Tucker, DVM1; David G. Wilson, DVM1; Shawn K. Reinink, MSc, PEng2; James L. Carmalt, VetMB, PhD1

1Department of Large Animal Clinical Sciences, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, SK, Canada
2Department of Mechanical Engineering, University of Saskatchewan, Saskatoon, SK, Canada

*Corresponding author: Dr. Tucker (michelle.tucker@usask.ca)

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OBJECTIVES
To characterize the 3-D geometry of the equine larynx replicating laryngeal hemiplegia and 4 surgical interventions by use of CT under steady-state airflow conditions. Secondly, to use fluid mechanic principles of flow through a constriction to establish the relationship between measured airflow geometries with impedance for each surgical procedure.

SAMPLE
10 cadaveric horse larynges.

PROCEDURES
While CT scans were performed, inhalation during exercise conditions was replicated for each of the following 5 conditions: laryngeal hemiplegia, left laryngoplasty with ventriculocordectomy, left laryngoplasty with ipsilateral ventriculocordectomy and arytenoid corniculectomy, corniculectomy, and partial arytenoidecction for each larynx while CT scans were performed. Laryngeal impedance was calculated, and selected cross-sectional areas were measured along each larynx for each test. Measured areas and constriction characteristics were analyzed with respect to impedance using a multilevel, mixed-effects model.

RESULTS
Incident angle, entrance coefficient, outlet coefficient, friction coefficient, orifice thickness, and surgical procedure were significantly associated with upper airway impedance in the bivariable model. The multivariate model showed a significant influence of incident angle, entrance coefficient, and surgical procedure on impedance; however, the orifice thickness became nonsignificant within the model.

CLINICAL RELEVANCE
Laryngeal impedance was significantly associated with the entrance configuration for each procedure. This suggested that the equine upper airway, despite having a highly complex geometry, adheres to fluid dynamic principles applying to constrictions within pipe flow. These underlying flow characteristics may explain the clinical outcomes observed in some patients, and lead to areas of improvement in the treatment of obstructive upper airway disease in horses.

Equine upper airway diseases that restrict airflow continue to present a complex problem for veterinary surgeons. Reported postoperative success rates range from 60 to 80%, with a trend toward improvement over time.1–4 Multiple investigations regarding the fluid mechanics of the equine upper airway have been performed to evaluate the effect of surgical interventions on air movement. Both in vivo and ex vivo studies5–10 have reported the impedance of the airway as an outcome measure to predict clinical improvement in patients. Specifically, equine recurrent laryngeal neuropathy (RLN) has been investigated with correlating measurements of the visual appearance of the larynxes, such as the amount of arytenoid abduction and the cross-sectional area (CSA) of the rima glottis.7,11–13 Measurement of the visible CSA of the rima glottis has been generally accepted but recent studies7,12,13 have reported different methodologies for camera placement and angulation. In 1 study,7 the distance from the camera was challenging to quantify given the varying laryngeal lengths within a standardized box. Ultimately, this introduces a potential source of error based on the measurement of a 2-D area from a 3-D structure and fails to address the effective flow area caudal to the rima glottis where ventricular dilation occurs. Rima glottis CSA measurement simulates the intraoperative endoscopic image of the larynx conformation during a laryngoplasty procedure but does not provide adequate understanding of
the collapse that may occur when the horse is exercising at maximal effort.\textsuperscript{7}

The previous study\textsuperscript{7} yielded questions regarding the geometry associated with the airways and the development of flow within them. In the discipline of fluid mechanics, airflow within a pipe with a constricted region has been studied to determine how the narrowed region (orifice) affects flow and pressure.\textsuperscript{3,15} Flow development for a given orifice is strongly correlated with energy loss and pressure drop, due to irregularities in the flow caused by the orifice shape.\textsuperscript{15} In human airways, idealized versions of the vocal folds demonstrated airflow separation, wherein the jet of air breaks into eddies as it emerges into an expanded area. This separation may explain clinical phenomena in these patients, such as changes in voice quality during speech.\textsuperscript{16,17} Similarly, some of the postoperative complications observed in equine patients and outcomes of ex vivo airway studies may be explained by fluid flow phenomena.

The objectives of this study were to characterize and report the 3-D geometry of the equine larynx while replicating left laryngeal hemiplegia (RLN), left laryngoplasty with ipsilateral ventriculocordectomy (LLP), left laryngoplasty with ventriculocordectomy combined with left arytenoid corniculecctomy (LLP-COR), left arytenoid corniculectomy (COR), and left partial arytenoidecction (PA) by use of CT while simulating inhalation during exercise. A second objective was to examine these geometries within the paradigm of fluid dynamic principles for airflow through constrictions within a circular pipe. The first hypothesis was that despite concurrent airflow and subsequent motion, CT images of sufficient quality could be captured for analysis. The second hypothesis was that the experimental impedance for various laryngeal configurations would correspond with constriction and divergence geometrical characteristics, namely, the angle of incidence, frictional loss factor, dimensionless orifice thickness, and inlet and outlet area ratios.

**Materials and Methods**

**Specimens**

Ten larynges from equine cadavers with no reported history of upper airway disease were collected as previously described.\textsuperscript{7} These were preserved in saline (0.9% NaCl) solution–saturated gauze wraps and frozen at –20 °C within 2 hours after euthanasia by placement in a freezer until the time of use. The larynges were thawed in a room at 23 °C for 20 hours to allow trimming of the excess tracheal rings and esophagus, leaving the cricoarytenoideus dorsalis muscles bilaterally intact prior to experimentation.

**Procedures**

An instrumented box of the exact dimensions and construction was used as reported previously and larynges were similarly mounted using a polyvinyl chloride pipe adapter with diameter of 2.5 or 3.81 cm with nylon cable ties, and a nail to anchor the epiglottis to a foam board during each testing period.\textsuperscript{7} Adapter size was chosen based upon the tightness of fit within the trachea. Separate differential pressure transducers were connected to polyurethane catheter tubing within the tracheal lumen (Model P55D; Validyne Engineering) and the inside of the box (Model DP103-14; Validyne Engineering). Each pressure was measured relative to the pressure within the room. An inlet airflow regulation valve was used upstream to adjust flow; steady flow was maintained during each testing period and was measured using an orifice plate according to ISO standard 5167 with a third differential pressure transducer (Model DP103-14; Validyne Engineering) to measure the pressure drop across the plate.\textsuperscript{18}

Airflow was adjusted by an assistant to blind the author to the pressure differentials generated by each laryngeal simulation, with the goal of a tracheal pressure of –4.3 kPa and maximal airflow of 70 L/s as has been reported for a horse at maximal exertion.\textsuperscript{19,20} Flow was adjusted to achieve the desired tracheal pressure using a downstream valve to regulate the vacuum. Airflow and pressure within the box and trachea were measured for each simulation for each larynx while the CT was performed. A USB analog-to-digital converter (USB-6221; National Instruments) was used to acquire data at 100 Hz, which were recorded using commercial software (LabView; National Instruments). The difference between the pressures was divided by the flow at the time to yield laryngeal impedance which was reported for each test. A mean value was calculated for each of these values over a 10-second period, given that flow and pressure were controlled in a steady-state fashion. Data were collected to obtain consistent measurements for at least 20 seconds corresponding with a CT scan, which was performed in conjunction with airflow (Supplementary Figure S1). Helical scans were performed at 1-mm slice thickness with settings of 120 kVp, 200 mAs, and 0.64-mm pitch on a machine (Toshiba Aquilon One; Canon Medical Systems) with a scan speed of 1 mm/s.

Each larynx underwent testing while replicating the disease state and then 4 different sequential surgical interventions. Left RLN (disease) was replicated by placing a nylon suture (Ethibond Excel; Ethicon US) from the right cricoïd cartilage to the arytenoid cartilage and allowing the left arytenoid cartilage to collapse under airflow. The cricoïd bite was taken 5 to 10 mm lateral to the sagittal ridge of the cricoïd cartilage, so that the suture would fall within a palpable notch when possible. The arytenoid bite was taken through the muscular process of the right arytenoid cartilage in a dorsomedial to ventrolateral direction. This was tied with a surgeon’s throw with 5 more throws to replicate maximal abduction of the right arytenoid cartilage. Each larynx was tested in this configuration, and then an LLP procedure was performed. A continuous pattern of 4-0 poliglecaprone 25 (Monocryl; Ethicon US) was used to close the left ventriculocordectomy incision. The left-to-right quotient (LRQ) was measured by capturing a digital still image of the lar-
larynx and measuring the angle from midline for both the left and right arytenoid corniculate processes, as has been reported. An LRQ of 0.85 to 0.95 was considered acceptable to replicate clinically acceptable abduction. The larynx was tested again, and then an LLPCOR procedure was performed by incising through the mucosa and cartilage, removing the corniculate process of the left arytenoid cartilage. The mucosal edges were adhered to the cartilage using cyanoacrylate glue to replicate postoperative healing. The larynx was tested and a COR procedure was subsequently performed by removing the body of the arytenoid cartilage as described by White et al., and the mucosal edges were glued. A final test with airflow was performed. With each test, a concurrent CT scan was performed.

Digital video using a USB camera (C920 Webcam; Logitech) was obtained for comparison with the CT images. Still frames were captured from those videos for analysis of the rima glottis areas as performed previously. Open-source software (ImageJ; National Institutes of Health) was used to perform the measurements using the right arytenoid cartilage to provide a scale along with the thyrohyoid width. Three measurements were taken of each rima glottis area excluding the saccules and averaged to represent the CSA as measured from the video for the RLN, LLP, LLPCOR, and COR states.

From each CT examination, the image was balanced within the transverse plane using the caudal aspect of the cricoid cartilage. Three separate areas were measured for each specimen based on the coronal plane cross section. The first area was measured where a complete ring of tissue was first present along the longitudinal axis of the larynx; this was comparable to the rima glottis area for the videos for analysis of the rima glottis areas as performed previously. Open-source software (ImageJ; National Institutes of Health) was used to perform the measurements using the right arytenoid cartilage to provide a scale along with the thyrohyoid width. Three measurements were taken of each rima glottis area excluding the saccules and averaged to represent the CSA as measured from the video for the RLN, LLP, LLPCOR, and COR states.

Figure 1—CT images of a cadaveric horse larynx in a recurrent laryngeal neuropathy state (left image) that shows convergence and divergence of the airflow pathway as well as corresponding first, second, and third cross-sectional areas where they fall on the axial section (right images). The large arrow at the top of the left image shows the direction of airflow and the rostral aspect of the larynx. A = Right arytenoid cartilage. C = Cricoid cartilage. V = Ventricles.
Statistical analysis

Data were imported into a commercial statistics package for analysis (Stata version 14.0; StataCorp). Variables were initially screened using bivariable analysis, and when the P value was < 0.2, they were subsequently considered in the multivariable analysis. A multilevel, mixed-effects model controlling for individual larynx was used using a backward stepwise approach to examine the effects of the significant variables on the outcome variable of impedance.

Results

A mean negative tracheal pressure of 4.3 kPA was maintained for each procedure (Table 1). During data analysis for each test for each larynx, a 10-second period of steady flow was chosen, and the mean value was used to reflect the measured tracheal and box (pharyngeal) pressures. For the LLP procedure, the LRQ ranged from 0.85 to 0.95, with a mean of 0.89. Predicted mean impedance by procedure was graphically displayed (Figure 3). The frictional loss coefficient in increasing order was LLP, LLPCOR, PA, COR, and RLN, which represented increasing amount of work done by the patient to breathe, respectively. The geometrical measurements, including the areas, incident angle, inlet and outlet ratios, and dimensionless orifice coefficients were also summarized (Table 2). The first and second measured areas showed the most variation between procedures, which coincided with a1, the ratio of these areas. The dimensionless orifice ratio, s/D, did not show any correlation in procedure order, compared with the other variables. The CT areas were plotted with the areas measured from the digital videos and showed a trend toward correlation.

All CT images captured allowed areas to be measured even with vibrations of the tissues noted during testing (Figure 1). Incident angle was exponentially associated with the logarithmic transformation of the impedance (Figure 3).

Table 1—Mean (range) airflow and measured pressures for cadaveric horse larynges in a recurrent laryngeal neuropathy (RLN) and 4 surgical (LLP, LLPCOR, COR, and PA) states.

<table>
<thead>
<tr>
<th>State</th>
<th>Airflow (L/s)</th>
<th>TP (kPa)</th>
<th>PP (kPa)</th>
<th>K</th>
</tr>
</thead>
<tbody>
<tr>
<td>RLN</td>
<td>20.2 (5.59–33.4)</td>
<td>4.34 (4.20–4.44)</td>
<td>0.43 (0.06–1.07)</td>
<td>18.4 (0.38–81.1)</td>
</tr>
<tr>
<td>LLP</td>
<td>39.3 (20.1–54.5)</td>
<td>4.37 (4.25–4.66)</td>
<td>1.38 (0.53–3.13)</td>
<td>1.88 (0.029–5.82)</td>
</tr>
<tr>
<td>LLPCOR</td>
<td>37.9 (14.2–53.5)</td>
<td>4.33 (4.27–4.53)</td>
<td>1.50 (0.29–3.01)</td>
<td>2.40 (0.091–8.01)</td>
</tr>
<tr>
<td>COR</td>
<td>22.5 (5.7–37.8)</td>
<td>4.30 (4.24–4.35)</td>
<td>0.53 (0.063–1.70)</td>
<td>12.5 (0.18–54.0)</td>
</tr>
<tr>
<td>PA</td>
<td>24.6 (12.54–35.7)</td>
<td>4.32 (4.26–4.38)</td>
<td>0.55 (0.22–1.00)</td>
<td>6.50 (0.18–28.6)</td>
</tr>
</tbody>
</table>

COR = Corniculectomy. K = Frictional loss coefficient. LLP = Left-sided laryngoplasty with ipsilateral ventriculocordectomy. LLPCOR = Left-sided laryngoplasty with ipsilateral ventriculocordectomy combined with corniculectomy. PA = Partial arytenoidectomy. PP = Pharyngeal air pressure. TP = Tracheal air pressure.

Data reported as the mean and range. The reported pressures are negative relative to atmospheric.
Table 2—Mean (range) geometric values for the larynges of Table 1 under airflow by procedure.

<table>
<thead>
<tr>
<th>State</th>
<th>$A_1$ (cm$^2$)</th>
<th>$A_2$ (cm$^2$)</th>
<th>$A_3$ (cm$^2$)</th>
<th>$\sigma_1$</th>
<th>$\sigma_2$</th>
<th>Angle (°)</th>
<th>s/D</th>
</tr>
</thead>
<tbody>
<tr>
<td>RLN</td>
<td>8.73 (5.70 to 11.23)</td>
<td>4.9 (1.924 to 8.56)</td>
<td>15.8 (10.51 to 19.29)</td>
<td>2.01 (1.01 to 3.23)</td>
<td>0.32 (0.14 to 0.70)</td>
<td>14.54 (0.24 to 28.47)</td>
<td>1.88 (1.25 to 2.70)</td>
</tr>
<tr>
<td>LLP</td>
<td>12.85 (5.70 to 21.84)</td>
<td>10.4 (5.66 to 21.30)</td>
<td>16.3 (11.44 to 20.88)</td>
<td>1.32 (0.61 to 2.40)</td>
<td>0.62 (0.29 to 1.02)</td>
<td>6.42 (–8.74 to 16.02)</td>
<td>1.35 (0.75 to 2.04)</td>
</tr>
<tr>
<td>LLP COR</td>
<td>12.77 (7.05 to 19.2)</td>
<td>9.8 (5.56 to 18.50)</td>
<td>16.0 (11.29 to 19.87)</td>
<td>1.51 (1.10 to 2.35)</td>
<td>0.60 (0.30 to 0.98)</td>
<td>10.53 (–1.54 to 10.08)</td>
<td>1.16 (0.69 to 1.91)</td>
</tr>
<tr>
<td>COR</td>
<td>8.77 (6.01 to 11.45)</td>
<td>5.3 (2.65 to 6.99)</td>
<td>15.4 (10.62 to 19.20)</td>
<td>1.72 (1.20 to 2.27)</td>
<td>0.35 (0.25 to 0.57)</td>
<td>16.17 (11.79 to 24.53)</td>
<td>1.39 (1.03 to 2.30)</td>
</tr>
<tr>
<td>PA</td>
<td>9.35 (8.10 to 10.92)</td>
<td>8.3 (4.51 to 13.90)</td>
<td>15.3 (10.6 to 18.95)</td>
<td>1.29 (1.10 to 1.38)</td>
<td>0.54 (0.4 to 0.86)</td>
<td>7.06 (–17.30 to 26.20)</td>
<td>1.02 (0.32 to 1.62)</td>
</tr>
</tbody>
</table>

A1, A2, and A3 = First, second, and third cross-sectional areas. D = Constriction diameter. s = Constricted region. $\sigma_1$ = Inlet coefficient. $\sigma_2$ = Outlet coefficient.

Figure 4—Predicted mean impedance by procedure, with 95% CIs. COR = Corniculectomy. LLP = Left-sided laryngoplasty with ipsilateral ventriculocordectomy. LLP COR = Left-sided laryngoplasty with ipsilateral ventriculocordectomy combined with corniculectomy. PA = Partial arytenoidectomy. RLN = Recurrent laryngeal neuropathy. $P < 0.05$ different from each other.

From the bivariable analysis, procedure, $\sigma_1$, $\sigma_2$, K, and s/D were significant ($P < 0.001$). These variables were included in the initial multivariable model, and then a backward elimination approach was used to determine which variables were significant with respect to impedance. The multivariable mixed-effects model showed a significant effect of procedure, incident angle, and inlet ratio on impedance. There was a residual intraclass correlation of 86%. Impedance was significantly different between the RLN state and the PA procedure, compared with the LLP and COR procedures, while the LLP COR was not significantly different from the other 4 states (Figure 4). Thus, the LLP and LLP COR procedures had the least resistance to airflow over the RLN and PA procedures.

Discussion

The box model has been previously published as a controlled method for comparing between degrees of abduction of the laryngoplasty, new surgical prototypes, and other upper airway procedures. Compared with the previous studies, similar magnitudes of impedance were reported for the replicated disease and procedures. The COR and LLP procedures had significantly lower impedance to airflow than the RLN state and the PA procedure in the present study. The friction coefficients reported for each procedure in Table 1 reflected previous findings, with increased energy loss associated with the RLN, COR, and PA procedures over the LLP and LLP COR procedures. This value reflects energy lost with air movement across the larynx and relates to the work done by the patient during breathing. Compared with previous research, the RLN impedance reported here was much higher but this may be explained by having different populations of larynges and a different airflow model (dynamic versus static). A static airflow model was necessary in this instance to ensure clear CT images for analysis.

Three-dimensional imaging affords greater insight into the diagnosis and treatment of upper airway obstructive disorders in horses. Using CT imaging and markers, 1 study found that the arytenoid cartilage moves about 3 axes during abduction, with the primary movement of interest due to rocking from the lateral belly of the cricoarytenoideus dorsalis muscle. More recently, Lynch et al related this motion to varying levels of force on the laryngoplasty suture while also replicating inhalation within a larynx. That report also demonstrated insignificant gains in arytenoid angle and airflow beyond approximately 50% of the maximal suture abduction force. Uniting the anatomical configuration of arytenoid abduction with the CSAs along the larynx and fluid mechanics will lead to a more accurate awareness of airflow as it relates to patient outcomes. Computed tomography, MRI, and transesophageal ultrasound have also led to insights about the pathology and diagnosis of RLN. Decreased cricoarytenoideus dorsalis muscle volume, muscle belly CSA, neuronal density and percentage of collagen and fat have all been associated with progressively decreased arytenoid abduction. While these efforts focused on diagnostic capabilities and decreasing cricoarytenoideus dorsalis muscle function, the present study demonstrated the possible therapeutic potential of these imaging modalities beyond diagnosis alone.

The literature reporting the box model concludes that while it does not exactly capture the clinical scenario, it does duplicate respiratory mechanics that have been experimentally confirmed in adult horses. Complete paralysis of the cricoarytenoideus dorsalis muscle as occurs with advanced RLN is successfully duplicated, and previous research found that measured CSA of the rima glottis is significantly associated with impedance. However, it raised some questions about the validity of this measure. The ventricles within the larynx lie caudal to the rima glottis, but may collapse with severe disease, further narrowing the effective CSA of airflow. Area measurements from both the CT images and digital video showed a trend toward correlation in the present study, but outlying values raised questions about why these differences in measurement might have
occurred. One consideration is that the arytenoid cartilages are angled differently within the coronal plane between larynges. Dependence upon a scale measurement within digital imaging software versus CT images may also influence measurement accuracy. As the ability to improve image analysis improves with technology, this is likely to be the more consistent methodology for future studies.

Form within the upper airway significantly affects function, especially with equine performance. While decreased CSA has been the primary focus, there are other factors that have not been examined previously, such as the length over which airway constriction occurs, and the outflow conformation. Within a constricted region of a pipe the ratio of the constriction to original pipe diameter, angle of the inlet, and thickness of the constriction are significantly associated with increasing turbulence in fluid mechanics. The area ratios observed here indicate that the level of constriction, relative to the patient’s initial pharyngeal and tracheal diameters, significantly affect impedance. The replicated disease and procedures in the present study demonstrated a thick orifice conformation (σ > 0.5). This means that airflow within the constricted portion of the larynx is expected to reattach to the walls before exiting into the trachea, which could increase wall stress experienced by the caudal larynx. In patients with a longer larynx, this equates to a greater propensity for collapse with higher velocities closer to the caudal portion of the arytenoid cartilages. Given the proposed cycle of collapse causing increased air velocity and negative pressure resulting in more collapse, this may represent a potential area of intervention. The incident angle of airflow demonstrated an exponential relationship with impedance, which is also expected for flow within a pipe. There is an ideal angle of approach to a narrowed region that reduces the amount of flow disturbance. Clinically this means that in a horse with a wide pharynx and a narrower trachea, a greater degree of abduction may be advantageous in creating a smoother transitional zone as air flows into the larynx. These regions are known to have a significant effect on flow development in fluid mechanics but have not been examined in the horse to the author’s knowledge.

In the human airway surgery literature, experimental simplified models based on actual patient conformations have been fundamental to understanding the fluid mechanics before incorporating complexities such as tissue elasticity, muscular function, and fluid-structure interactions. The areas, ratios, and orifice thickness reported in this paper may be used to develop similar models for horses. By contrast, the vocal folds of humans have been extensively researched due to their direct influence on speech quality, but unanticipated impacts on airflow were also discovered. Recirculation regions and flow separation were observed in both symmetrically and in asymmetrically angled models. These recirculating areas may contribute to more stress on the larynx due to increased velocity profiles. Given that within the human airway these regions represent a significant constriction, the same may be true in a horse with RLN such that the airflow in the region is heavily influenced by small conformational changes. The present study demonstrated that the point of maximal constriction occurs at the level of the ventricles which likely play a significant role in arytenoid collapse. The 2000 study by Jansson et al also revealed that a laryngoplasty with cordopexy did reduce the drop in pressure and increased the airflow across the larynx, but this was not found to be significant. In cases of inadequate abduction, removal of the ventricle and vocal fold may therefore improve laryngeal CSA and airflow. Further investigation regarding the role of the ventricle in equine airflow is warranted.

The present study reported a 3-D, stressed image of the upper airway that is unlikely to be possible in equine patients for the foreseeable future. It sets the foundation for basic configurational analysis of the equine upper airway; however, there are many areas where further investigation is needed. The laryngoplasty with or without a corniculectomy improved impedance significantly over the RLN state, and the fluid mechanics reported here would suggest that a corniculectomy may be advantageous in horses with an elongated laryngeal region. Additionally, creating a smooth “funneling” transition from pharynx to trachea should be prioritized to maximize postoperative airflow while minimizing the work of breathing.

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The authors declare that there were no conflicts of interest.

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References


Supplementary Materials

Supplementary materials are posted online at the journal website: avmajournals.avma.org