Dynamic respiratory endoscopic findings pre- and post laryngoplasty in Thoroughbred racehorses

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Summary

Reasons for performing study: To investigate laryngeal function in cases of recurrent laryngeal neuropathy pre- and post laryngoplasty (LP) using dynamic respiratory endoscopy (DRE).

Objectives: To compare the rima glottidis area during DRE pre- and post LP; document all forms of dynamic upper airway obstruction (DUAO) pre- and post LP and investigate the relationship between post operative abduction at rest and exercise.

Study design: Prospective case series.

Methods: Thoroughbred racehorses with pre- and post LP DRE were included. Left-to-right arytenoid cartilage angle ratios and rima glottidis area ratios were used to quantify laryngeal function during rest and exercise, pre- and post LP.

Results: In 35 horses, mean pre-LP ratios were greater during rest (left-to-right quotient angle ratio [LRQ] 0.76 ± 0.13; left-to-right arytenoid ratio [LRR] 0.72 ± 0.14; rima glottidis area ratio [RGA] 0.40 ± 0.10) than exercise (LRQ 0.39 ± 0.16, P < 0.001; LRR 0.38 ± 0.16, P < 0.001; RGA 0.15 ± 0.05, P < 0.001). Exercising ratios were larger post LP (LRQ 0.61 ± 0.13; LRR 0.60 ± 0.12; RGA 0.30 ± 0.08) than pre-LP (LRQ 0.39 ± 0.16, P < 0.001; LRR 0.38 ± 0.16, P < 0.001; RGA 0.15 ± 0.05, P < 0.001). A positive linear relationship was found between post LP resting and exercising ratios (R² = 0.48, P < 0.001) and post operative abduction grades (R² = 0.63, P < 0.001). Pre-LP all horses demonstrated left arytenoid cartilage collapse with bilateral vocal cord collapse and 37% had aryepiglottic fold collapse. Post LP 13 horses (37%) developed additional DUAOs and these horses had smaller RGAs (0.25 ± 0.08) than horses that did not develop DUAOs (0.32 ± 0.07, P = 0.01).

Conclusions: Multiple forms of DUAO occurred pre-LP and additional forms often developed post LP. Post LP the degree of arytenoid cartilage abduction at rest was useful to predict the degree of abduction during exercise.

Keywords: horse; laryngoplasty; endoscopy; rima glottidis; exercise

Introduction

Recurrent laryngeal neuropathy (RLN) is a performance limiting condition in the equine athlete which becomes clinically apparent during exercise when arytenoid cartilage abduction cannot be sustained against increasingly negative inspiratory pressures [1]. Prosthetic laryngoplasty (LP) aims to increase and maintain the glottic cross-sectional area by abducting and stabilising the arytenoid cartilage, thereby preventing dynamic collapse during exercise [2].

Dynamic respiratory endoscopy (DRE) either overground [3] or on a treadmill [4] can be used to definitively diagnose dynamic upper airway obstructions (DUAO) during high-speed exercise. Recently using DRE, 3 studies identified multiple forms of DUAO in 71–87% of horses post LP [5–7] including palatal dysfunction, aryepiglottic fold collapse and vocal cord collapse. However, it is not known whether these complex forms of DUAO were present prior to LP or developed following surgical intervention. Submaximal arytenoid cartilage abduction may predispose horses to develop additional DUAOs [8]. The development of additional forms of DUAO following LP in a series of horses has not been investigated.

The gold standard for objective evaluation of upper airway surgery in racehorses is measurement of upper airway flow mechanics during exercise [9–12]. However, the required methodology is not readily adaptable in a clinical setting. Ratios of arytenoid cartilage abduction have been used experimentally to evaluate the effect of different surgical treatments on the upper airway [13,14] and have been described during DRE to assess the variability in arytenoid cartilage position during exercise [15,16]. An aim of this study was to describe an objective, noninvasive and clinically applicable method to quantify the cross-sectional area of the rima glottidis during exercise.

The objectives of this study were to: 1) Compare left arytenoid cartilage abduction and the rima glottidis area during DRE in horses pre- and post LP, 2) document all forms of DUAO that occur pre- and post LP and 3) determine if post operative left arytenoid cartilage abduction at rest can be used to predict the degree of abduction during exercise.

We hypothesise that there will be a significant increase in the rima glottidis area during exercise post LP compared with pre-LP; that no additional forms of DUAO will develop following LP and the degree of left arytenoid cartilage abduction at rest post LP can predict position during exercise.

Materials and methods

Case selection

All Thoroughbred racehorses that underwent an LP and bilateral ventriculocordectomy (VCE) at Randwick Equine Centre with a pre- and post operative DRE between June 2011 and June 2013 were included in the study. The post operative DRE was offered at no cost to the owner. Horses were in full race training at the time of both examinations.

Resting and dynamic respiratory endoscopy

An overground videendoscopy unit (Dynamic Respiratory Scope V2)a was used for all endoscopic examinations [3] with electronics attached to a custom-made saddle pad. Horses wore their normal racing gear, a global positioning device and heart rate monitor (Polar Equine RS800CX G3)b. The endoscope was introduced via the right nares and positioned in the pharynx to ensure the rima glottidis and both corniculate processes were clearly visible.

Prior to each exercising examination, a resting endoscopic examination of the laryngeal airway was performed with the horse induced to swallow on several occasions using the endoscopic water flush. Exercising examinations were performed on circular racecourses as a continuous episode of high-intensity exercise to replicate ‘race-like’ conditions, as recommended by previous studies [17]. A typical exercise test consisted of a 1000 m trot, 800 m canter, followed by 1000 m of gallop exercise. Average speed over the final 1000 m and final 200 m of high-intensity exercise; maximal heart rate and total distance travelled were recorded.

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Post operative DREs were performed after return to full race training and the exercise intensity was replicated as close as possible to the preoperative examination.

Surgical procedure

Horses underwent general anaesthesia and a left-sided LP without modification was performed by one of the authors (J.M.L.) [1]. The cricopharyngeus and thyropharyngeus muscles were separated along their aponeurosis to expose the muscular process of the arytenoid cartilage and 2 sutures of number 5 silicone-coated braided polyester (Ticro) were placed between the caudal aspect of the cricoid cartilage and muscle process of the arytenoid cartilage. Intraoperative videendoscopy (Olympus CF Q140) was performed to achieve 80–90% of maximal arytenoid abduction [8,18]. A bilateral VCE via a laryngotomy approach was then performed [1] by everting then sharply excising each laryngeal ventricle, then excising part of each vocal fold from 3 mm proximal to the ventral junction of thyroid cartilage wings to the vocal laryngeal ventricle, then excising part of each vocal fold from 3 mm to 5 mm to the apex of the corniculate processes (COR) [22] were also recorded. Digitalised still images were captured from all pre- and post operative DREs and Video analysis and image capture

Dynamic respiratory endoscopy video recordings were reviewed by both authors using multimedia software (QuickTime Pro). Resting laryngeal function score [20] or post operative arytenoid abduction grade [18] were determined prior to exercise. Arytenoid cartilage position during exercise was graded pre [16] and post operatively [18]. The presence of vocal cord collapse (VCC) [21], intermittent dorsal displacement of the soft palate (DDSP) [21], axial deviation of the aryepiglottic folds (ADAF) [19] and ventral displacement of the apex of the corniculate processes (COR) [22] were also recorded.

Laryngeal ratios

Three laryngeal ratios were calculated for each image. All ratios were calculated 3 times by the same observer using image analysis software (ImageJ) and the mean value used in the statistical analysis:

1) Left-to-right quotient angle ratio (LRQ). A line drawn from the ventral to dorsal aspect of the rima glottidis is extended by one third and used to measure the left (L°) and right (R°) arytenoid angles, where LRQ is equal to L° divided by R° (Fig 1a) [13].

2) Left-to-right arytenoid angle ratio (LRR). A line half as long as the right arytenoid angle is extended dorsally from the apex of the right corniculate and used to measure to the left (L°) and right (R°) angles, where LRR is equal to L° divided by R° (Fig 1b) [15].

3) Rima glottidis area ratio (RGA). The luminal margins of the rima glottidis are digitally traced to calculate the cross-sectional area. The length of the rima glottidis from its ventral aspect to the apex of the right corniculate process is measured. The RGA is the rima glottidis area divided by the rima glottidis length squared (Fig 1c). This novel ratio was created to analyse the effect of all forms of DUAO on the rima glottidis area. In a pilot study, this method produced similar ratio values with minimal variance when the same image was measured at increasing distance intervals from the endoscope tip (mean ± s.d.: 0.15 ± 0.005) and no linear relationship was found between increasing distance and RGA value (R² = 0.06, J.L. Leutton, unpublished data).

Data analysis

Results are presented as mean ± s.d. Data analysis was performed using commercial statistical software (IBM SPSS Version 19.0). All statistical assumptions were tested prior to interpretation of the results. Paired t tests were used to compare laryngeal ratios at rest and exercise, pre- and post operatively and exercise intensities. Student’s t test was used to compare the proportion of the population with ADAF pre- and post operatively. Logistic regression was used to assess if pre- and post operative abduction grade could be used to predict the development of DUAOs and the development of complications. Simple linear regression was used to investigate the relationship between post operative abduction at rest and during exercise. Statistical significance was set at P<0.05.

Results

Of 49 horses that underwent a LP and VCE with a preoperative DRE within the time frame of the study, 35 horses underwent a post operative DRE. Post operative DRE was not performed on the other 14 horses due to illness/injury unrelated to the surgical procedures (n = 5); sale of the horse (n = 2); retired to stud (n = 2); trainer/owner declining a follow-up DRE (n = 2).
and surgical complications (n = 3). The mean number of days between date of surgery and the post operative DRE was 184 days, ranging from 104 to 364 days. There were 7 females (20%), 11 entire males (31%) and 17 geldings (49%) age 2–7 years (3.4 ± 1.0 years). All horses had a history of abnormal respiratory noise during strenuous exercise and 25 horses (71%) were reported to exhibit exercise intolerance.

**Exercise tests**
There was no significant difference between pre- and post operative exercise in total distance travelled 2600 ± 296 m vs. 2654 ± 317 m (P = 0.2), average speed over the final 1000 m 15.1 ± 0.8 m/s (P = 0.6) and average speed over the final 200 m 16.9 ± 1.0 m/s (P = 0.9). Due to equipment malfunction, complete heart rate data was not available in 12 horses. There was no significant difference in maximal heart rates during preoperative (218 ± 12.4 beats/min) and post operative exercise tests (220 ± 10.3 beats/min, P = 0.4) for 23 of the 35 horses. The set of differences between observations for each paired t test, and those described below, were found to be normally distributed.

**Comparison of laryngeal ratio methods**
Preoperative evaluation during exercise revealed a strong positive correlation between LRQ and LRR values (R² = 0.99; P < 0.0001) and agreement between the 2 measurement techniques was demonstrated on a Bland–Altman plot, with an average difference from the mean of 0.005 ± 0.014 (95% CI -0.02 –0.03) (Supplementary item 2) and good agreement between the 2 measurement techniques was demonstrated on a Bland–Altman plot, with an average difference from the mean of 0.005 ± 0.014 (95% CI -0.02 –0.03) (Supplementary item 3). There was a moderate positive correlation between LRQ and RGA values (R² = 0.33; P < 0.001) with 33% of the variability in RGA explained by the arytenoid angle ratio. The statistical assumptions for the aforementioned linear regression models and those described below were tested and found to be valid, including normality of the data and linearity, normality and homoscedasticity of the residuals.

**Dynamic upper airway obstructions**
Arytenoid cartilage abduction: Preoperatively, laryngeal function at rest was graded III-1 in 3 horses (9%), III-2 in 22 horses (63%), III-3 in 9 horses (26%) and IV in 1 horse (3%) [20] and arytenoid cartilage collapse (ACC) during exercise was graded as moderate in 7 horses (grade B, 20%) and severe in 28 horses (grade C, 80%) [16]. Preoperative mean resting laryngeal ratios (LRQ 0.76 ± 0.13; LRR 0.72 ± 0.14; RGA 0.40 ± 0.10) were significantly larger than exercising ratios (LRQ 0.39 ± 0.16; P < 0.001; LRR 0.38 ± 0.16; P < 0.001; RGA 0.15 ± 0.05; P < 0.001) (Supplementary item 4).

Post operatively, arytenoid cartilage position at rest was grade II in 12 horses (34%), grade III in 14 horses (40%), grade IV in 7 horses (20%) and grade V in 2 horses (6%) [18]. Subjectively, there was no change in post operative abduction grade between rest and exercise in 22 horses (63%); loss of one grade of abduction in 11 horses (31%) and loss of 2 grades of abduction in 2 horses (6%). There was a significant positive linear relationship between the degree of post operative arytenoid cartilage abduction during rest and exercise, using both LRQ values (R² = 0.48; P < 0.001, Fig 2) and the post operative abduction grade (R² = 0.63; P < 0.001) [18].

Objectively, mean post operative laryngeal ratios at rest [LRQ 0.68 ± 0.13; LRR 0.68 ± 0.13; RGA 0.38 ± 0.09] were significantly larger than during exercise [LRQ 0.61 ± 0.13; P < 0.001; LRR 0.60 ± 0.12; P < 0.001; RGA 0.30 ± 0.08; P < 0.001] (Supplementary item 5). Comparison of mean laryngeal ratios during exercise revealed post operative ratios [LRQ 0.61 ± 0.13; LRR 0.60 ± 0.12; RGA 0.30 ± 0.08] to be significantly larger than preoperative ratios [LRQ 0.39 ± 0.16; P < 0.001; LRR 0.38 ± 0.16; P < 0.001; RGA 0.15 ± 0.05; P < 0.001] (Fig 3).

Axial deviation of the ADAF: Preoperatively, 13 horses had right-sided ADAF (Table 1). There was no significant difference in the mean laryngeal ratios at exercise between the 13 horses with preoperative ADAF (LRQ 0.41 ± 0.14; LRR 0.16 ± 0.05; RGA 0.39 ± 0.14) vs. the 22 horses without LRQ 0.40 ± 0.14, P = 0.9; LRR 0.14 ± 0.06, P = 0.4; RGA 0.37 ± 0.17, P = 0.8; LRQ: P = 0.899; LRR: P = 0.758; RGA: P = 0.384). The grade of ACC was unable to predict the likelihood of ADAF (χ² = 0.12, P = 0.7). Post operatively, 17 horses had ADAF (Table 1) [19]. Of the 6 horses that underwent laser resection of the right aryepiglottic fold, 3 horses demonstrated no evidence of ADAF during post operative DRE, one horse demonstrated no change in the degree of ADAF and 2 horses demonstrated a progression in the severity of ADAF (both displayed severe bilateral ADAF post operatively). All 7 horses with ADAF preoperatively that did not have laser resection of the fold, demonstrated ADAF on post operative DRE, with one horse developing bilateral ADAF. There was a significant increase in mean exercising LRQ and RGA values post LP (LRQ 0.65 ± 0.1; LRR 0.65 ± 0.09) compared with pre-LP (LRQ 0.38 ± 0.09, P = 0.001; LRR 0.36 ± 0.09, P = 0.002) in these 7 horses. Excluding horses that underwent laser resection of the aryepiglottic fold, horses were significantly more likely to have ADAF post LP compared with pre-LP (P = 0.02). The grade of post operative abduction during rest [18] did not predict the presence or absence of ADAF post operatively (χ² = 1.99, P = 0.4).

Development of DUAO post LP: Thirteen horses (37%) developed additional forms of DUAO post LP (Table 1, 5 unilateral ADAF, 5 bilateral ADAF, 4 COR, 1 DDSP; 2 horses developed 2 DUAOs). These horses had significantly
Discussion

This study used objective and clinically applicable methods to evaluate laryngeal function during high-intensity exercise before and after LP and VCE. The laryngeal ratios demonstrate a significant increase in the degree of left arytenoid cartilage abduction and the rima glottidis area during exercise following LP and agree with the improvement in upper airway flow mechanics post LP shown experimentally [9]. However, the ratios also indicate a significant degree of arytenoid instability between rest and high-speed exercise following LP, agreeing with the subjective loss of at least one grade of post operative abduction in 37% of horses in our study and 21–43% of horses elsewhere [5,23]. A modified LP procedure to promote ankylosis of the cricoarytenoid joint and improve arytenoid stability has been evaluated in a small sample of horses at rest [14]. Horses in the modified LP group had a significantly greater degree of arytenoid cartilage abduction at 3 months post operatively and lower translaryngeal impedance in vitro compared with horses undergoing traditional LP. Furthermore, in another study, horses returned to a similar level of performance after modified LP compared with their untreated cohorts [24]. The authors' are not aware of any previous exercising endoscopic comparison of the 2 LP techniques in clinical cases.

Preoperatively, bilateral VCC was identified in all horses in this study and those exercised on an incline at maximal intensity following left recurrent laryngeal neurectomy [25]. Interestingly, a much lower prevalence (22%) of bilateral VCC in combination with ACC was reported elsewhere [21], which may reflect differences in horse populations or be a function of exercise intensity. Although the maximal speed, total distance travelled and incline were not reported in the latter study, comparable exercise tests have been associated with slower speeds during treadmill DRE compared with overground DRE [17].

Following LP and VCE, collapse of vocal cord remnants occurred in 23% of horses in this study and has been reported elsewhere [5–7,21], highlighting the importance of complete vocal fold resection. Furthermore, it demonstrates the value of DRE as part of a complete and thorough evaluation of outcome following upper airway surgery.

The prevalence (37%) of preoperative ADAF in combination with ACC in our study was similar to the 29–35% reported by others [21,25]. The predominance of right-sided ADAF observed has been previously noted [19] and was proposed to be secondary to altered airflow and/or visual perspective due to placement of the videendoscope in the right nasal passage. Other possible aetiological factors contributing to ADAF include altered airway dynamics secondary to other forms of DUAOs [26], immaturity of mucosal tissue or cartilage and fatigue [19], submaximal arytenoid cartilage abduction [8] and laryngeal airway turbulence [27].

Almost half of the cases (48%) had ADAF post operatively concurring with the reported prevalence rates of 34–60% post LP [5–7]. Interestingly, horses were significantly more likely to have ADAF post LP compared with preoperatively, suggesting the surgical procedure may have a causative role in the development of ADAF, or that onset of the disease process occurs in the time frame between both exercising examinations. Additionally, ADAF may not be related to negative inspiratory airway pressures, a theory supported by our data because ADAF persisted post LP in all 7 horses with preoperative ADAF that did not undergo laser resection, despite significant improvement in their exercising laryngeal ratios post LP. Given the prevalence of ADAF pre- and post LP in the current study and elsewhere [5–7,21,25,26], there is strong support for preoperative assessment of upper airway function during exercise and potentially routine resection of the right aryepiglottic fold concurrently with the LP procedure if post operative DRE is not available [8]. Post operative DRE identified persistent ADAF in 50% of horses following laser resection, so potentially excision of a larger section of aryepiglottic fold tissue may decrease the incidence of ongoing ADAF. However, the authors are unaware of any studies documenting the extent of ADAF following laser excision.

Left-sided COR during DRE was present in 6% of our population preoperatively and 17% post operatively and has also been reported elsewhere in horses with RLN [21] and following LP [5,21]. Potential aetiological factors for COR include lack of dorsal arytenoid cartilage support from the left arytenoideus transversus muscle or an atypical transversus muscle or an atypical
manifestation of RLN [21]. The observed axial collapse may also reflect abnormal biomechanical properties of the elastic cartilage of the cornuicate process that is potentially exacerbated by point fixation following LP.

Unsurprisingly, horses that developed additional forms of DUAO had significantly smaller mean RGAs than horses that did not, therefore their presence may limit future athletic performance. Horses that developed additional forms of DUAO could not be predicted by their post operative abduction grade at rest and we were not able to demonstrate that, exercising LRO and LRR were lower in these horses compared with horses without additional DUAs. The lack of significance in this finding may be explained by the small study population. An experimental study suggested submaximal arytenoid cartilage abduction may predispose horses to other forms of DUAO, as it created areas of increased negative inspiratory pressure over both vocal cords, the right aryepiglottic fold and soft palate [8]. To reduce the likelihood of developing additional forms of DUAO post LP we recommend aiming for almost maximal abduction of the arytenoid cartilage at the time of the LP procedure in racehorses. In our population, a greater degree of arytenoid cartilage abduction post LP was not associated with an increased risk of dysphagia or reflux, supporting the findings of one study [23] and contradicting others [18,28].

The significant predictive relationship between the degree of post operative left arytenoid cartilage abduction at rest and during exercise using subjective and objective data, contradicts 2 studies that reported no relationship between the degree of post operative abduction at rest and the likelihood of ACC during exercise, except in horses with grade 5 post operative abduction [5,23]. The lack of association between rest and exercise in both of these previous studies may reflect their mixed-breed study populations and the variable exercise intensities performed, compared with the semi-standardised exercise tests performed on Thoroughbred racehorses in the current study.

An unexpected finding was reflux of white viscous fluid from the cranial oesophageal opening in 23% of horses during exercise post LP that was not present prior to surgery. Upper oesophageal incompetence following LP has recently been described during exercise [7,29], with iatrogenic damage to the caudal pharyngeal constrictor muscles, their innervation, the intrinsic oesophageal musculature, or surrounding adventitia hypothesised to be potential causes. The results of our study and those of Barakzai et al. [2014] indicate oesophageal reflux post LP occurs more commonly during exercise compared with rest, therefore it may explain the scant reports of this complication in the literature [29].

Potential study limitations of the current study include horses with surgical complications that were unable to resume race training and caudal positioning of the endoscope in the pharynx reducing detection of palatal instability. A potential limitation of the LRO and LRR techniques may occur if there is a loss of right arytenoid cartilage abduction, as this would result in an increase in the ratio value. Furthermore, the ratio techniques do not predict abnormal biomechanical properties of the elastic cartilage of the cornuicate process. The lack of significance in this finding may be explained by the small study population. An experimental study suggested submaximal arytenoid cartilage abduction may predispose horses to other forms of DUAO, as it created areas of increased negative inspiratory pressure over both vocal cords, the right aryepiglottic fold and soft palate [8].

Conclusions
Measurement of clinically applicable laryngeal ratios supported our hypothesis that the rima glottidis area during exercise was significantly increased following LP in our population of Thoroughbred horses. Multiple forms of DUAs occur commonly preoperatively; they will persist following LP if left untreated and additional forms of DUAs are likely to develop subsequent to LP. We found a relationship between subjective and objective measures of the degree of post operative left arytenoid cartilage abduction at rest and during exercise.

Authors’ declaration of interests
No competing interests have been declared.

Ethical animal research
Owners gave informed consent for their horses’ inclusion in the study.

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Authorship
J.L. Leutton and J.M. Lumsden contributed to the study design, data collection, data analysis and interpretation and manuscript preparation.

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References


Supporting information
Additional Supporting Information may be found in the online version of this article at the publisher’s website:

Supplementary Item 1: Upper airway videoendoscopic images of a horses with RLN preoperatively during rest and exercise (top left and right) and the same horse post LP and VCE during rest and exercise (bottom left and right).

Supplementary Item 2: Correlation of LRQ and LRR values, obtained from preoperative endoscopic images of the upper airway during high-speed exercise in 35 racehorses with RLN.

Supplementary Item 3: Bland–Altman plot of the data obtained from 35 paired arytenoid angle ratios measured using the LRQ and LRR methods during exercise preoperatively.

Supplementary Item 4: Preoperative laryngeal ratios (LRQ, LRR and RGA, mean ± s.d.) calculated from upper airway videoendoscopic images in horses with RLN (n = 35), between rest and exercise.

Supplementary Item 5: Post operative laryngeal ratios (LRQ, LRR and RGA, mean ± s.d.) calculated from upper airway videoendoscopic images in horses with RLN (n = 35), between rest and exercise.