Developing a new tool for detection of subclinical recurrent laryngeal neuropathy in horses

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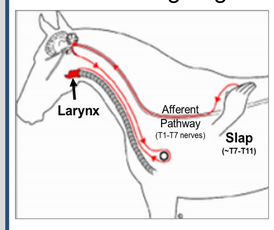
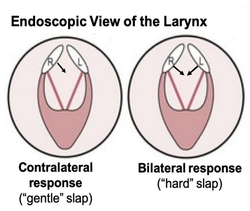
Introduction

Figure 1: Recurrent laryngeal neuropathy (RLN) causes laryngeal paralysis and airway obstruction that can end a racehorse's career.

- Recurrent laryngeal neuropathy (**RLN**) is an idiopathic distal axonopathy that commonly prevents maximal laryngeal dilation in athletic horses, thus limiting exercise performance **(Figure 1).**
- RLN diagnosis relies on *subjective grading* via endoscopic assessment of the larynx in conjunction with the **Slap Test** (**Figure** 2) to elicit the *thoraco-laryngeal adductor reflex* (**TLAR**) and/or through rigorous exercise.





Grade	Description
I	All arytenoid cartilage movements are synchronous and symmetrical. Full arytenoid cartilage abduction can be achieved and maintained.
II	Arytenoid cartilage movements are asynchronous and/or larynx is asymmetrical at times, but full arytenoid cartilage abduction can be achieved and maintained.
III	Arytenoid cartilage movements are asynchronous and/or asymmetric. Full arytenoid cartilage abduction cannot be achieved and maintained.
IV	Complete immobility of the arytenoid cartilage and vocal fold.

Figure 2: Slap Test and Subjective Grading Scale. The Slap Test is conducted by slapping the saddle region on each side of the horse to evoke the thoraco-laryngeal adductor reflex (TLAR). A gentle slap elicits a contralateral response while a firmer slap elicits a bilateral response. Laryngeal movement is graded using one of many Likert scales, such as the Havemeyer endoscopic laryngeal grading system shown above. *Adapted from: https://veteriankey.com/neurology/* and [1].

- The value of young racehorses is significantly influenced by the outcome of subjective grading, hence the need for an objective evaluation method.
- *In humans,* laryngeal function is evaluated by delivering *calibrated puffs of air directly to the laryngeal mucosa* to evoke the laryngeal adductor reflex via the superior (cranial) laryngeal nerve (**Figure 3**) [2]; henceforth referred to as the *cranial laryngeal adductor reflex* (**CLAR**) for comparison with the TLAR.

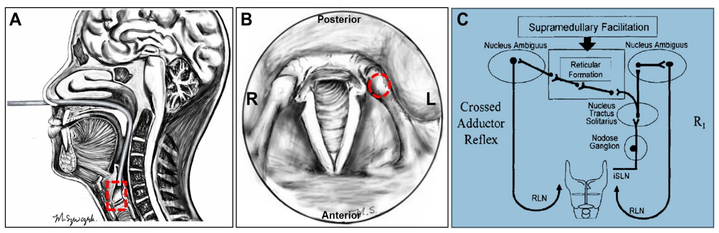


Figure 3: Human Laryngeal Function Testing. Transnasal endoscopic approach **(A)** in awake (unanaesthetized) humans to deliver calibrated air puffs directly to the laryngeal mucosa overlying the arytenoid cartilage **(B)**, which is innervated by afferents of the superior (cranial) laryngeal nerve **(C)**. Unilateral stimulation evokes a bilateral response. *Adapted from* Lever Lab Art (A & B) and [3] C).

- Our group has developed **laryngeal tracking software** to permit *objective quantification* of the CLAR in humans and rodent models [4,5].
- We also have developed an investigational device capable of producing high pressure air pulses to the equine larynx to test the CLAR for comparison with the TLAR.
- Our ultimate goal is to identify which reflex (TLAR or CLAR) is optimal for detection of *subclinical RLN* in horses.

Hypotheses

- *Air pulse stimulation of the larynx using our investigational device will reliably evoke the CLAR in horses.*
- *Laryngeal adductor reflex durational measures (obtained using our laryngeal tracking software) will not differ between the CLAR and TLAR due to recruitment of the same efferent pathway.*
- *Equine CLAR durational measures will be similar to those evoked in healthy young adult people.*

Methods

Transnasal Endoscopic Approach

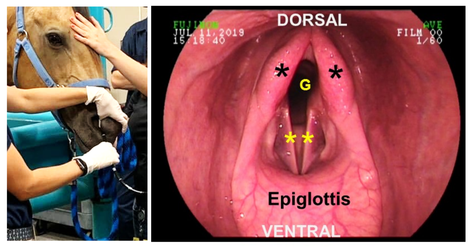


Figure 4: Transnasal Endoscopy to Visualize the Equine Larynx. Labels: yellow asterisks = vocal folds; black asterisks = corniculate processes; G = glottis.

- Testing is currently underway with healthy adult horses (n=10, either sex) from our Research Herd.
- Horses are tested while standing in stocks, without anesthesia **(Figure 4).**
- For each horse, the endoscope is inserted through the naris (either side) and slowly advanced to visualize the larynx **(Figure 4).**
- TLAR is tested first, immediately followed by CLAR testing (both described below).
- The entire procedure is video recorded at 30 frames per second.

Slap Test to Elicit the Thoraco-Laryngeal Adductor Reflex (TLAR)

- Each horse is gently slapped on the saddle region caudal to the withers, first on the left then right side.
- Stimulus pressure is subjectively categorized as either gently/light slapping to evoke a contralateral response (i.e., arytenoid adduction) or hard slapping to evoke a bilateral response (i.e., bilateral arytenoid adduction).
- On each side of the horse, 3-5 light slaps are immediately followed by 3-5 hard slaps, with a 3-5 second pause between stimuli.

Air Puffs to Elicit the Cranial Laryngeal Adductor Reflex (CLAR)

- Using our investigational device **(Figure 5)**, air puffs are delivered to 2 different laryngeal locations via PE240 catheter tubing fed through the endoscope working channel (**Figure 6**) to determine which location most reliably evokes the CLAR.
- Multiple (3-5) air puffs are delivered to each target location for both sides of the larynx.
- The distance between the catheter tip and the larynx is calculated by advancing catheter until it contacts the corniculate process at end of each endoscopic session; this step is important for determining air pressure.

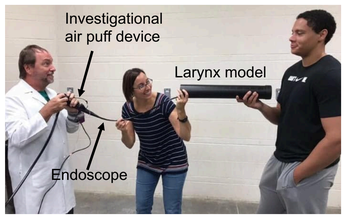


Figure 5: Bench Testing. Prior to testing, endoscopic maneuvering was practiced using a "plastic model".

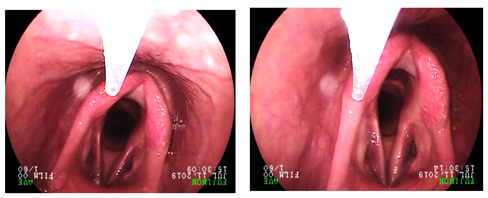


Figure 6: Anatomical Targets for Air Pulse Delivery. The catheter is shown aiming at the two target sites for air pulse delivery: the mid-corniculate process (**left image**) and the junction between the corniculate process and aryepiglottic fold (**right image**).

Preliminary Findings

- Testing has been completed for 2 of the 10 horses thus far; both horses tolerated the entire procedure well without sedation.
- Multiple TLAR and CLAR responses were evoked in both horses and accurately tracked using our laryngeal tracking software (see **Figure 7**).
- Delivering air puffs to the junction of the corniculate process and aryepiglottic fold most reliably evoked the CLAR.

Figure 7: Videos of Equine TLAR and CLAR. Left: contralateral and bilateral TLAR responses to gentle and hard Slap Tests. Right: a CLAR in response to air puff stimulation of the larynx.

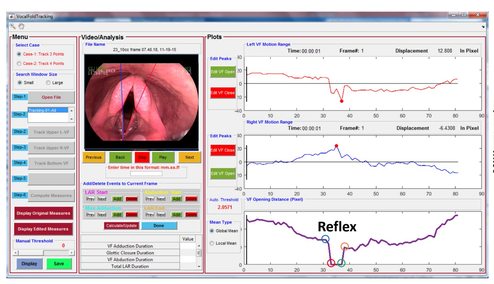


Figure 8: Laryngeal Tracking Software. Our proprietary software tracks the change in distance between the vocal folds or corniculates/arytenoids during TLAR/CLAR testing. Bottom graph combines right and left laryngeal movements to permit quantification of laryngeal adductor reflex measures. Circles denote the 4 durational measures shown in **Figure 9**.

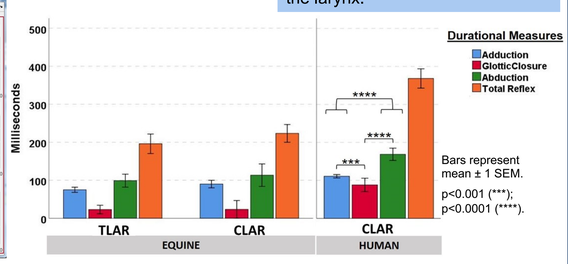


Figure 9: Cross-species Comparison of the Laryngeal Adductor Reflex. Left: results of the TLAR and CLAR from 2 horses. Right: human CLAR durational events from 20 healthy adults. Preliminary results suggest that TLAR and CLAR may not differ substantially since they do have the same efferent pathway. Also, equine CLAR appears to be faster (66%) than the human reflex.

Anticipated Results

- Characterize durational measures associated with objective TLAR and CLAR outcomes in a cohort of healthy adult horses.
- Compare results between TLAR and CLAR protocols.
- Ascertain minimal effective pressure needed to elicit the CLAR in horses.
- Determine the optimal working distance for endoscopic placement with respect to the larynx.
- Determine whether the equine CLAR is indeed faster than its human equivalent.

Limitations

- Limited sample size.
- Horse demeanor.
- Uncontrolled timing/pressures applied during TLAR.
- Timing of air puff to inspiration/expiration.
- Lacking *post-mortem* affirmation of absence of disease.

Future Directions

- Report equine testing outcomes along with comparison to other species.
- Investigate objective CLAR and TLAR outcomes in horses with laryngeal disease.
- Determine the effect of tranquilizer administration on CLAR and TLAR outcomes in healthy horses.
- Explore the extent of TLAR responsiveness beyond the saddle region.
- Create a TLAR slapping device to minimize user variance and standardize pressures and timing.
- Explore the effect of head position on laryngeal function.

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Acknowledgments

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