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**Article:**

Barakzai, Safia, Wells, Jeremy, Parkin, T. et al. (2018) Overground endoscopic findings and respiratory sound analysis in horses with recurrent laryngeal neuropathy after unilateral laser ventriculocordectomy. *Equine Veterinary Journal*. ISSN: 2042-3306

<https://doi.org/10.1111/evj.12993>

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1 **Overground endoscopic findings and respiratory sound analysis in horses with recurrent**  
2 **laryngeal neuropathy after unilateral laser ventriculocordectomy**

3  
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9  
10 **Ethical considerations:** Informed client consent was obtained for all horses included in this  
11 study

12  
13 **Competing interests:** None

14  
15 **Sources of Funding:** Horserace Betting Levy Board Small Project Grant

16  
17 **Acknowledgements:** Dr Chris Baldwin for performing RGA ratio and LRQ measurements.

18  
19 **Authorship:**

20 S. Z. Barakzai: study design, data collection and study execution, data analysis and  
21 interpretation, preparation of manuscript

22  
23 J. Wells: Data analysis and interpretation, preparation of the manuscript

24  
25 T. Parkin: Statistical analysis, preparation of manuscript

26  
27 P. Cramp: study design, data analysis and interpretation, preparation of the manuscript

28 **Overground endoscopic findings and respiratory sound analysis in horses with recurrent**  
29 **laryngeal neuropathy after unilateral laser ventriculocordectomy**

30

31 **Summary**

- 32 • **Background:** Unilateral ventriculocordectomy (VeC) is frequently performed, yet  
33 objective studies in horses with naturally occurring RLN are few.
- 34 • **Objectives:** To evaluate respiratory noise and exercising over-ground endoscopy in  
35 horses with grade B and C laryngeal function, before and after unilateral laser VeC.
- 36 • **Study Design:** Prospective study in clinically affected client-owned horses.
- 37 • **Methods:** Exercising endoscopy was performed and concurrent respiratory noise was  
38 recorded. A left sided laser VeC was performed under standing sedation. Owners were  
39 asked to present the horse for re-examination 6-8 weeks post-operatively when  
40 exercising endoscopy and sound recordings were repeated. Exercising endoscopic  
41 findings were recorded, including the degree of arytenoid stability. Quantitative  
42 measurement of left-to-right quotient angle ratio (LRQ) and rima glottidis area ratio  
43 (RGA) were performed pre- and post- operatively. Sound analysis was performed, and  
44 measurements of the energy change in F1, F2 and F3 formants between pre- and post-  
45 operative recordings were made and statistically analysed.
- 46 • **Results:** Three grade B and 7 grade C horses were included. 6/7 grade C horses pre-  
47 operatively had bilateral vocal fold collapse (VFC) and 5/7 had mild medial deviation of  
48 the right ary-epiglottic fold (MDAF). Right VFC and MDAF was still present in these  
49 horses post-operatively. Sound analysis showed significant reduction in energy in

50 formant F2 (P=0.05) after surgery. Ongoing left arytenoid instability, right VFC and  
51 MDAF caused continued noise.

52 • **Main Limitations:** The study sample size was small and multiple dynamic abnormalities  
53 made sound analysis challenging.

54 • **Conclusions:** RLN-affected horses have reduction of sound levels in F2 after unilateral  
55 laser VeC. Continuing noise may be caused by other ongoing forms of dynamic  
56 obstruction. Unilateral VeC is useful for grade B horses based on endoscopic images. In  
57 Grade C horses, bilateral VeC, right ary-epiglottic fold resection +/- laryngoplasty might  
58 be a better option than unilateral VeC.

59

## 60 **Introduction:**

61 To most owners, trainers, and referring veterinarians, reduction of respiratory noise is a key  
62 factor when determining whether or not an upper respiratory tract surgery has been  
63 'successful'. Respiratory noise has shown an association with other objective measures of  
64 upper airway parameters, such as trans upper airway inspiratory pressures, and is therefore  
65 a useful measure of surgical success<sup>2</sup>. Unilateral ventriculocordectomy (VeC) is probably one  
66 of the most frequently performed upper respiratory tract surgeries, yet there are no  
67 publications that quantitatively analyse its effect on respiratory sound production in horses  
68 with naturally occurring recurrent laryngeal neuropathy (RLN), including in horses with  
69 grade B exercising laryngeal function<sup>1</sup> (Table 1) and vocal fold collapse. Two previous  
70 experimental studies have found that bilateral VeC (via laryngotomy) or unilateral  
71 transendoscopic laser VeC restore sound levels close to baseline (pre-neurectomy levels) in  
72 grade 4 horses<sup>2,3</sup> which is rather surprising, given that the left arytenoid is presumably still  
73 collapsing during inspiration, causing respiratory obstruction and turbulent airflow. A clinical

74 study of draft horses with grade 4 laryngeal function<sup>4</sup> (left-sided hemiplegia) found that  
75 bilateral surgical VeC significantly reduced inspiratory noise, but these horses were only  
76 exercised at a trot<sup>4</sup>.

77

78 Furthermore, no previous study has evaluated horses both pre- and post-VeC using  
79 exercising endoscopy, to ascertain if the surgery has resulted in stabilisation of the left  
80 arytenoid, or if other dynamic respiratory abnormalities are present. We aimed to evaluate  
81 exercising over-ground endoscopic videos (both subjectively and objectively) and  
82 respiratory sound production in horses before and after unilateral VeC, including horses  
83 with both grade B and grade C laryngeal function at exercise<sup>1</sup>.

84

#### 85 **Materials and Methods:**

86 This study was a prospective clinical trial. A power calculation using sound analysis data  
87 from previously published studies<sup>2,3</sup> revealed a sample size of 3 horses from each of grades  
88 B and C exercising laryngeal function would be necessary for the study to have 80% power.  
89 Client-owned horses with naturally occurring RLN were recruited into the study. Informed  
90 client consent was obtained from all owners.

91

92 Pre-operatively, exercising over-ground endoscopy was performed in all horses using a  
93 Videomed Overground Scope<sup>a</sup> to confirm the diagnosis of RLN (grade B or C exercising  
94 laryngeal function plus vocal fold collapse (VFC) +/- right medial deviation of the ary-  
95 epiglottic fold (MDAF), and to exclude horses which had other forms of dynamic upper  
96 respiratory collapse. The exercise test was tailored to the horses' usual mode of work, and  
97 the rider wore a GPS watch to record the speed and duration of exercise. A recording of

98 respiratory noise was made during the test with a unidirectional cardioid microphone  
99 (E608, Sennheiser <sup>b</sup>), which was attached to the endoscope using Velcro straps, and  
100 positioned at the right nostril. The microphone was connected to a digital recorder (DR40  
101 handheld 4-track recorder, Tascam <sup>c</sup>) which was placed in the saddle-pad of the over-ground  
102 endoscope.

103

#### 104 *Laser surgery:*

105 Horses were pre-medicated with flunixin meglumine (1.1 mg/kg IV, Flunixin Injection,  
106 Norbrook <sup>d</sup>), butorphanol (0.1 mg/kg IV, Butador, Chanelle <sup>e</sup>), and procaine benzyl penicillin  
107 (12 mg/kg IM, Depocillin, MSD animal health <sup>f</sup>). Animals were then sedated with romifidine  
108 (0.08 mg/kg IV, Sedivet, Boehringer Ingelheim Vetmedica <sup>g</sup>). Xylazine (0.4 mg/kg IV,  
109 Virbaxyl, Virbac <sup>h</sup>) was used to provide additional sedation if required.

110

111 Sedated horses were positioned in stocks and a left sided VeC was performed with a diode  
112 laser (VetArt 980 Diodenlaser <sup>i</sup>) under video-endoscopic guidance. The caudo-medial wall of  
113 the left ventricle was first grasped and the mucosa of the ventricle partially everted using  
114 60cm long Equine Laryngeal Forceps <sup>j</sup> before the everted portion was excised using the  
115 diode laser with a power setting of 15W (continuous wave). The laryngeal forceps were  
116 then repositioned on the mid-section of the medial edge of the left vocal cord and the cord  
117 was excised using the laser at the same setting, using the method described by Henderson  
118 *et al.*<sup>5</sup>. Briefly, 2 horizontal cuts were made to transect the dorsal and ventral attachments  
119 of the vocal cord. A vertical cut was then made from dorsal to ventral to free the cord from  
120 its attachment to the lateral larynx. After the cord was excised, the laser surgery site was  
121 sprayed topically via a trans-endoscopic catheter with approximately 7ml of 2mg/ml

122 dexamethasone solution (Dexadreson, Dechra<sup>k</sup>). Total laser energy used was not recorded  
123 as the laser unit utilized in this study did not generate this information.

124

125 Horses were discharged from the hospital an hour or so after surgery, when they had  
126 sufficiently recovered from sedation to travel. They were starved for 4 hours after surgery  
127 to allow the effects of topical local anaesthetic to wear off. A 10 day course of  
128 phenylbutazone (2 mg/kg BID PO for 5 days then 2mg/kg SID PO for 5 days, Equipalazone<sup>k</sup>)  
129 was prescribed. Topical 'throat spray' was not administered. Owners were instructed to  
130 box rest horses for the first week and then to turn out in a small paddock for 3 weeks before  
131 re-commencing normal ridden work. Owners were also asked to return the horse at 6-8  
132 weeks post-operatively for a re-evaluation.

133

134 At the post-operative evaluation, exercising over-ground endoscopy and respiratory noise  
135 recordings were repeated in the same manner as pre-operatively.

136

137 *Analysis of exercising endoscopy videos:*

138 Videos were assigned a random number and were analysed in a blinded manner by a single  
139 ECVS diplomate with significant experience in interpreting exercising endoscopy. The  
140 presence of standard dynamic upper airway abnormalities<sup>6</sup> was recorded. Additionally, for  
141 each video, the left arytenoid cartilage was graded as stable, mildly unstably or markedly  
142 unstable<sup>7</sup> when the horse was exercising maximally.

143

144 Three freeze frames of each video were obtained from both pre- and post-operative  
145 endoscopic examinations, taken at a time when the respiratory obstruction was deemed to

146 be maximal. From these, left-to-right quotient angle ratio (LRQ) and rima glottidis area ratio  
147 (RGA) (Figure 1) were calculated as described previously by Leutton and Lumsden<sup>8</sup> using  
148 image analysis software (Image J<sup>1</sup>). The mean values from the 3 freeze framed images were  
149 calculated for each horse pre- and post-operatively. Descriptive analysis of this data was  
150 described. Further statistical analysis of LRQ and RGA values is not presented as no power  
151 calculation had been performed for this data.

152

### 153 *Respiratory sound analysis*

154 From each pre- and post-operative sound recording, a 10 second section of respiratory  
155 noise was taken when the horse was exercising at maximal effort, near to the end of the  
156 exercise test. A semi-automated approach to formant identification and measurement was  
157 adopted and implemented in Matlab<sup>m</sup>. This provided a consistent basis for making  
158 comparisons between cases and more details of this methodology are provided in  
159 Supplementary Item S1. All recordings were high-pass filtered (using a finite impulse  
160 response filter of order 50) with a lower cut-off frequency of 200 Hz, to reduce the effects of  
161 low frequency 'rumble' due to hoof noise.

162

163 Inspiratory breaths were identified using a semi-automated approach (see S1). The  
164 duration of inspiration that was used for analysis was taken as being from 0.1 seconds  
165 before to 0.1 seconds after the peak of energy within each inspiration. Formants were  
166 identified using Linear Predictive Coding analysis (see S1), with the search for formant peaks  
167 constrained to the following previously described<sup>9</sup> frequency regions: F1 = 0 - 600 Hz, F2=  
168 900 - 2400 Hz and F3= 2800 - 4800 Hz. Within each of these regions the centre of the  
169 frequency analysis bin with highest local maximum energy was taken as being the centre of

170 the formant. To measure the sound energy within each formant, a 2048-order third-octave  
171 filter centred on the formant's central frequency was derived. The total amount of energy  
172 within the third-octave bands for each of ten inspiratory breaths was then summed. The  
173 relative total energy (dB) for each formant (F1, F2, F3) for each case was calculated as:  $10 \times$   
174  $\log_{10}$  [summed energy from 10 inspirations].

175

176 Statistical analyses were performed using Minitab. Pre- and post-operative total sound  
177 energy values (dB) for each formant were compared using Wilcoxon Sign ranked test. Mann  
178 Whitney tests were used to compare between grade B and C horses for pre-operative sound  
179 energy levels, post-operative sound energy levels and reduction in sound energy levels.

180 Statistical significance was set at  $P \leq 0.05$ .

181

## 182 **Results:**

183 Ten horses were included in the study; mean age was 6 years (range 3-15 years). Their use  
184 was: show-jumping (4), hunting (3), dressage (1), eventing (1), and National Hunt racing (1).  
185 From GPS data, the maximal speed attained (mean 19.7 km/h) and total distance cantered  
186 over (mean 1.6 km) were very similar for pre- and post-operative exercise tests for 9 horses  
187 that were cantered either in a ménage or in a field. One racehorse was only cantered at  
188 18.6 km/h pre-operatively as it was not deemed fit enough to gallop, but post-operatively it  
189 was galloped at 38 km/h.

190

191 Pre-operatively, at rest, 1 horse had grade 3.1 laryngeal function, 5 had grade 3.2, 2 had  
192 grade 3.3 and 2 had grade 4 laryngeal function<sup>1</sup>. At exercise, 3 had grade B and 7 had grade  
193 C laryngeal function. All grade B horses had stable left arytenoid cartilages and only left

194 sided VFC. Of the 7 grade C horses, 4 left arytenoid cartilages were deemed to be markedly  
195 unstable, 2 were mildly unstable and 1 was deemed to be stable. Six of 7 grade C horses had  
196 bilateral VFC, and 5/7 had mild right-sided MDAF (figure 2).

197

198 Surgery was completed without peri-operative complications in all cases. Horses were re-  
199 presented for follow-up examination at a mean of 8.5 weeks post laser surgery (range 6-16  
200 weeks).

201

202 *Post-operative endoscopic findings (Supplementary items 2-4):*

203 Nine of 10 laser surgery sites had healed fully (figure 3) at the time of re-examination. One  
204 horse that represented at 6 weeks post-operatively had a roughened edge to the laser  
205 surgery site caused by small granuloma formation (figure 3). These granulomas were  
206 removed under sedation with a trans-endoscopic diode laser (10W continuous wave) and  
207 the horse discharged with a further 7 day course of phenylbutazone (2 mg/kg BID PO for 3  
208 days then 2mg/kg SID PO for 4 days, Equipalazone<sup>k</sup>). This horse was re-examined 3 weeks  
209 later when the site appeared smooth (figure 3) and the post-operative exercise test,  
210 endoscopy and sound recording were performed at this time.

211

212 Post-operative exercising endoscopy (supplementary items 2-4) revealed ongoing right VFC  
213 (figure 4) in 6/7 grade C horses, and one grade B horse also showed mild right VFC. All 5  
214 grade C horses that had mild right-sided MDAF pre-operatively continued to exhibit the  
215 same degree of MDAF post-operatively. Arytenoid cartilage stability at exercise appeared,  
216 subjectively, to be partially improved (from severely unstable to mildly unstable) after  
217 unilateral laser VC in 3 grade C horses (Supplementary item 4).

218

219 A summary of LRQ and RGA data is shown in Table 2. As would be expected, horses with  
220 grade B laryngeal function had larger LRQs and RGA ratios than those horses with grade C  
221 laryngeal function both pre- and post-operatively. Although statistical tests were not  
222 performed on these endoscopically generated data, for all horses, the mean pre- and post-  
223 operative LRQs were similar (0.58 +/- 0.19 pre-op vs 0.57 +/- 0.19 post-op), indicating that  
224 the degree of arytenoid abduction was similar before and after surgery. The post-operative  
225 RGA ratios for all horses (mean = 0.28 +/- 0.10) were consistently larger than their pre-  
226 operative RGA ratios (mean = 0.24 +/- 0.11).

227

228 *Sound analysis:*

229

230 *Subjective description of pre-operative spectrograms:*

231 All 3 Grade B horses had quite a different appearance of their spectrograms in the F2  
232 formant frequency range compared to the 7 grade C horses. In grade B horses, the energy  
233 in F2 was generally less intense, and the band of high intensity 'abnormal' sound was  
234 confined to a much narrower frequency range within F2 (figure 5). This band of sound was  
235 approximately 500 Hz wide (range 450-550 Hz) and was contained within the upper half of  
236 F2 in all horses (centred at a mean of 1970 Hz, range 1725-2200 Hz). An abnormal band of  
237 sound could not be identified in the previously defined F3 formant frequency range (2800-  
238 4800 Hz) in any grade B horse (figure 5).

239

240 In grade C horses pre-operatively, inspiration was frequently louder than expiration, and the  
241 band of 'abnormal' inspiratory sound energy was spread right across the frequency range of  
242 F2 (900 - 2400 Hz, figure 6). In 3/7 grade C horses, all of which were graded as having  
243 markedly unstable left arytenoid cartilages, there was visibly increased sound energy in the  
244 F3 formant frequency range (2800-4800 Hz, figure 6). However in 4/7 grade C horses, 3 of  
245 which had mildly unstable arytenoids and 1 of which had a markedly unstable arytenoid,  
246 there was only very mild or no visibly increased sound energy in the F3 formant  
247 range. Although 6/7 grade C horses had MDAF, this could not be identified specifically  
248 within the spectrograms, probably because the whole of the F2 formants of these horses  
249 contained high levels of energy.

250

251 *Objective analysis of audio files:*

252 Pre-operatively, grade B horses (mean 23.9dB, range 17.5-29.9dB) had lower sound energy  
253 values in the F2 formant of inspiration than grade C horses (mean 31.6dB, range 27.8-  
254 34.3dB), but this finding was not statistically significant ( $P=0.07$ ). Post-operatively there was  
255 also no significant difference ( $p=0.25$ ) in sound energy values within the F2 formant of  
256 inspiration between grade B (mean 16.9 dB, range 15.1-18.1dB) and C (mean 22.7dB, range  
257 14.2-33.3) horses.

258

259 The mean post-operative reduction in the energy within the  $1/3^{\text{rd}}$  octave band in F2 was -  
260 8.3dB (range -0.3- -13.3, SD 4.3), in F1 was 1.08 dB (range +18 - -8.9, SD 7.8), and in F3 was -  
261 2.3 (range +3.7 - -8.42, SD 4.1). Only the reduction in sound intensity of F2 was found to be  
262 statistically significant ( $P=0.05$ ). Reduction in F2 sound intensity was not significantly  
263 different between horses with grade B or grade C exercising laryngeal function ( $P=0.27$ ).

264

265 **Discussion:**

266 Unilateral VeC is one of the most common upper respiratory tract (URT) surgeries  
267 performed on performance horses, yet there is limited evidence available in the veterinary  
268 literature regarding this surgical technique. The major aims of VeC surgery are to reduce  
269 respiratory noise at exercise and improve ventilatory parameters in horses with RLN and  
270 VFC, however it is the absence of noise that is most commonly judged by owners, trainers  
271 and veterinary surgeons as the major measurement of 'success' following URT surgery.

272

273 Laser VeC is a minimally invasive procedure that has gained widespread popularity. It is  
274 commonly performed unilaterally on the left vocal fold either with or without concurrent  
275 laryngoplasty and is often performed with right sided ventriculectomy. No previous studies  
276 have objectively analysed unilateral laser VeC for treatment of vocal fold collapse in horses  
277 with naturally occurring RLN. Two previous studies performed in horses with  
278 experimentally induced grade 4 RLN have found that bilateral VeC (performed with a  
279 scalpel) or unilateral left laser VeC both restore sound levels to close to baseline (pre-  
280 neurectomy levels) in grade 4 horses<sup>2,4</sup>. This finding is rather surprising, given that all grade  
281 4 horses can be assumed to have grade C laryngeal function<sup>10,11</sup>, and in such horses, the left  
282 arytenoid is presumably still collapsing during inspiration post-VeC, causing ongoing  
283 respiratory obstruction and turbulent airflow. It has been suggested that the VeC procedure  
284 may, in some way, stabilize a previously unstable left arytenoid cartilage thus reducing the  
285 noise<sup>3</sup>, but this theory has not been supported with endoscopic evidence. A clinical study of  
286 grade 4 RLN affected draft horses found that bilateral surgical VeC significantly reduced  
287 inspiratory noise, but these horses were only exercised at a trot (mean speed 4.6m/s)<sup>4</sup>. It

288 would be fair to say that horses that are exercised at the canter and gallop are likely  
289 generate greater trans-tracheal negative pressures and therefore experience more severe  
290 degrees of arytenoid collapse than those exercised at a trot.

291

292 In naturally occurring cases of RLN, which are presented for surgery, a spectrum of laryngeal  
293 dysfunction can be observed, including ipsilateral VFC in conjunction with varying severities  
294 of arytenoid collapse. Additionally, horses with naturally occurring RLN appear to have a  
295 high prevalence of MDAF and right VFC. We do not know if these abnormalities occur in  
296 horses with experimentally induced RLN, but we assume that they might not. All published  
297 studies thus far that have objectively analysed sound production in horses with RLN have  
298 only included Havemeyer grade 4 (exercising grade C ) horses<sup>2-4,9</sup>, and this study is the first  
299 to perform sound analysis in horses with a range of resting (grade 3.1, 3.2, 3.3 and 4) and  
300 exercising (grades B and C) laryngeal function.

301

302 *Equipment set up:*

303 In the present study the microphone was placed close to the right nostril, similar to the  
304 method previously reported by Derksen *et al*<sup>9</sup>. In contrast to Derksen *et al.*'s<sup>9</sup> methodology  
305 where the microphone was attached to a cavesson noseband, we attached the microphone  
306 to the insertion tube of the over-ground endoscope, with the microphone positioned at the  
307 level of the right nostril. To assess if the presence of the scope had an effect on sound  
308 recordings, we performed a pilot study recording respiratory noise in 2 horses which were  
309 exercised with the scope in place, and then again without the scope in place but with the  
310 microphone attached via the bridle in a similar position. There was no discernible effect of  
311 the insertion tube of the endoscope being in place when sound recordings were made.

312 Additionally, in the current study, both pre- and post-operative recordings were made with  
313 the endoscope in place, so the presence of the endoscope should not have had any effect  
314 on comparison of spectrograms and objective measurements of sound.

315

#### 316 *Timing of re-evaluations*

317 Although owners were asked to return their horses 6-8 weeks after surgery, there was some  
318 variation in timing of re-evaluation (mean 8.5 weeks, range 6-16 weeks) which is an  
319 unfortunate consequence of conducting a study in client-owned horses. Horses that were  
320 examined after a longer interval included the horse that had a second laser surgery at 6  
321 weeks post-operatively, and several which were put out to grass for a prolonged rest period  
322 after surgery, thus were not fit enough to perform the exercise test at the designated 6-8  
323 weeks. Previous studies have shown that the post-operative time period can have a  
324 variable effect on sound production between 60 and 120 days after VeC<sup>2,3</sup> and this may  
325 have had a small effect on our results.

326

#### 327 *Videoendoscopic findings*

328 In this study 6/7 horses with grade C laryngeal function had bilateral VFC recognised when  
329 exercising endoscopic videos were carefully analysed. Bilateral VFC has also been reported  
330 in 35/35 of horses with naturally occurring RLN that subsequently underwent laryngoplasty  
331 <sup>8</sup>. Although some surgeons must have suspected this for some time, because they routinely  
332 advocate bilateral VeC<sup>2,12</sup>, it is still common to only remove the left vocal fold in horses with  
333 RLN<sup>13-15</sup>. The current study and that of Leutton and Lumsden<sup>8</sup> suggest that bilateral vocal  
334 fold collapse is very common in horses with RLN. Equine surgeons may be wary of  
335 performing bilateral VeC because of the perceived risk of inducing ventral laryngeal webbing

336 and stenosis, which can occur whether using a scalpel blade or a laser<sup>16</sup>. Aggressive  
337 bilateral VeC in association with laryngoplasty may also predispose horses to post-operative  
338 food aspiration and coughing (N. Ducharme, personal communication). Safer alternatives to  
339 full bilateral VeC include using a scalpel rather than a laser, leaving the ventral 5mm of each  
340 vocal cord in situ and therefore preserving the vocal fold fornix, suturing the edge of the  
341 fold to the axial border of the ventricle<sup>15</sup>, only removing the dorsal half of the right vocal  
342 fold, or performing a right vocal cordotomy (rather than cordectomy) to induce scarring in  
343 the right vocal fold and thus reduce the severity of right VFC (F. Rossignol, personal  
344 communication).

345

346 Right sided MDAF has been previously reported in horses with RLN both pre-<sup>8</sup> and post-  
347 laryngoplasty<sup>8,17,18</sup>. If present pre-operatively, it should be addressed at the time of  
348 surgery. In our group of horses, the severity of right sided MDAF was mild in all cases, both  
349 pre- and post-operatively. The contribution of this minor degree of MDAF to respiratory  
350 obstruction is likely to be quite small, and its contribution to abnormal respiratory noise is  
351 unknown as there are no publications describing sound analysis of horses with MDAF.

352 Based on previously published studies, its relatively high prevalence post-operatively after  
353 LP<sup>8,17,18</sup> might even lend weight to the practice of routine removal of the right ary-epiglottic  
354 fold in all horses undergoing laryngoplasty surgery.

355

356 The degree of left arytenoid abduction did not appear to be much changed after left laser  
357 VeC, as evidenced by very similar pre- and post-operative LRQs. Rima glottis area was  
358 slightly increased by the surgery in almost all horses, with grade C horses tending to have a  
359 larger increase, presumably because the left VFC was more obstructive in these cases

360 compared to grade B horses. The small number of horses in this study should be considered  
361 when evaluating such small changes in endoscopic measurements. Subjectively, laser VeC  
362 appeared to partially improve the stability of the left arytenoid in 3/7 grade C horses that  
363 pre-operatively had marked instability of this structure, and these results support the theory  
364 first postulated by Robinson *et al.*<sup>3</sup>.

365

### 366 *Sound analysis*

367 A semi-automated method of sound analysis was developed for this study, where the total  
368 energy contained within a 1/3 octave band centred around the peak of energy within F2  
369 was calculated. We believe that this semi-automated method should be more accurate  
370 than previously reported methods which relied on visual inspection of the spectrogram to  
371 detect a single 'peak' of energy within each formant<sup>2-4</sup>. It is possible that evaluating the  
372 energy in the entire F2 frequency range (900-2400Hz) might better detect differences  
373 between grade B and C horses, and horses with stable and unstable arytenoid cartilages,  
374 rather than restricting evaluation to the energy within a 1/3 octave band.

375

376 Previous studies have analysed the sound spectrum of horses with experimentally induced  
377 (grade 4/4) RLN<sup>2,3,9</sup>, which would all be expected to have grade C laryngeal function at  
378 exercise<sup>10,11</sup>, but spectral analysis of grade B horses with vocal fold collapse has not  
379 previously been reported. Only 3 grade B horses were included in this study, but  
380 subjectively, visual analysis of their spectrograms demonstrated a much narrower abnormal  
381 band (approximately 500 Hz wide) of sound energy observed during inspiration, compared  
382 with the broad band of increased energy within F2 seen in horses with grade C laryngeal  
383 function. Certainly, horses with a stable, partially abducted arytenoid and vocal fold

384 collapse make a higher pitched inspiratory noise often described as a 'whistle', whereas  
385 those with complete collapse of the arytenoid are often described as making a louder, lower  
386 pitched inspiratory 'roar'. In this study it would appear that the spectrograms reflect this  
387 difference in audible abnormal sound in clinical cases. Additionally, abnormal sound energy  
388 could not be visually identified in the F3 formant in grade B horses.

389

390 It has recently been proposed that an additional grade, 'D', of exercising laryngeal function  
391 should be introduced to differentiate between a minimally abducted but relatively stable  
392 left arytenoid and one that dynamically collapses into the contralateral rima glottidis during  
393 inspiration<sup>19</sup>. If the current study had included a larger number of horses, we believe it is  
394 likely that it would be possible to make a differentiation between grades C and D using  
395 sound analysis, as the 'noisiest' spectrograms and audio files were clearly from horses which  
396 had markedly unstable grade C (the proposed grade 'D') arytenoids. Three out of 4 grade C  
397 horses with markedly unstable arytenoids were the only horses that had visibly increased  
398 sound energy within the F3 formant. We therefore suggest that increased sound energy  
399 within the F3 formant may be associated with active collapse of the arytenoid cartilage as is  
400 seen in the proposed grade D horses. It is likely that the absence of abnormal sound energy  
401 in the F3 formant in 7/10 horses in this study (pre-operatively) would explain why no  
402 statistically significant difference was found when comparing F3 sound levels pre- and post-  
403 operatively.

404

405 Although the energy in the F2 formant was statistically significantly reduced after laser VeC  
406 when the group of 10 horses was analysed, a good proportion of horses (particularly  
407 unstable grade C horses) still made an audible abnormal inspiratory noise after surgery

408 (supplementary items 3 and 4). This illustrates that just because a clinical measurement has  
409 statistically significantly improved, a clinically obvious abnormality may still be present.  
410 Continuing abnormal respiratory noise after VeC was not caused by incomplete resection of  
411 the fold and collapse of left vocal fold remnants, but was more likely attributable to other,  
412 often pre-existing, dynamic obstructions including continuing arytenoid instability in grade C  
413 horses, and/or right VFC and MDAF. The F2 formant has been identified as the formant  
414 that most closely reflects noise associated with collapse of the left vocal cord, ventricle and  
415 corniculate process and body of the arytenoid cartilage<sup>2,3,4</sup>. However, it is not known which  
416 frequency ranges abnormal noise created by other collapsing structures (such as MDAF)  
417 would lie within and experimental models to evaluate these do not exist, to our knowledge.  
418 This would be an interesting area of further research.

419

#### 420 *Conclusions*

421 This study was limited by its small sample size and the fact that multiple dynamic disorders  
422 made sound analysis challenging. It has, however, highlighted several findings that are of  
423 clinical relevance to horses with RLN: firstly, that in horses with Grade C laryngeal function,  
424 bilateral VFC and right sided MDAF are extremely common. Secondly, that horses with  
425 grade B laryngeal function and VFC make significantly less noise and have a narrower band  
426 of abnormal energy in the F2 formant as compared to horses with grade C laryngeal  
427 function and arytenoid cartilage collapse. Thirdly, this study also suggests that in some  
428 cases, laser VeC can stabilise a previously unstable arytenoid cartilage to some degree.  
429 Finally, due to continued right VFC, right MDAF and most importantly, continuing arytenoid  
430 instability, unilateral laser VeC is not necessarily a useful treatment option for horses with  
431 unstable grade C laryngeal function at exercise. This is especially true if the clinical

432 resolution of respiratory sound is the main objective of surgery. Bilateral VeC or  
433 laryngoplasty plus VeC +/- right ary-epiglottic fold resection may be a better option for  
434 horses with grade C RLN.

435

#### 436 **Manufacturers' addresses**

437 <sup>a</sup> Videomed GmbH , Munich, Germany

438 <sup>b</sup> Sennheiser UK Ltd., Marlow, UK

439 <sup>c</sup> TEAC UK Ltd, Guildford, UK

440 <sup>d</sup> Norbrook, Corby, UK

441 <sup>e</sup> Chanelle UK, Hungerford, UK

442 <sup>f</sup> MSD animal health, Milton Keynes, UK

443 <sup>g</sup> Boehringer Ingelheim Vetmedica, Bracknell, UK

444 <sup>h</sup> Virbac, Bury St Edmunds, UK

445 <sup>i</sup> Jørgen Kruuse A/S, Langeskov, Denmark

446 <sup>j</sup> Karl storz GmbH & Co. KG, Tuttlingen, Germany

447 <sup>k</sup> Dechra Veterinary Products, Shewsbury, UK

448 <sup>L</sup> National Institutes of Health, Bethesda, Maryland, USA.

449 <sup>m</sup> MathWorks, Massachusetts, USA

450

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508

509 **Supporting information:**

510 Supplementary item 1: Detailed methodology of sound analysis.

511 Supplementary item 2: grade B horse pre and post-op

512 Supplementary item 3 : grade C pre- and post op with severely unstable arytenoid both pre-  
513 and post-op

514 Supplementary item 4: grade C pre- and post-op with severely unstable arytenoid pre-op  
515 which is partially stabilised post-op.