1 **Overground endoscopic findings and respiratory sound analysis in horses with recurrent**
2 **laryngeal neuropathy after unilateral laser ventriculocordectomy**
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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
18
19 **Authorship:**
20 S. Z. Barakzai: study design, data collection and study execution, data analysis and
21 interpretation, preparation of manuscript
22
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24
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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. 6/7grade 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[2]. 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[1] (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[2,3] 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[4] (left-sided hemiplegia) found that

75   bilateral surgical VeC significantly reduced inspiratory noise, but these horses were only

76   exercised at a trot[4].

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[1].

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[2,3] 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[a] 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 cardiodid microphone

99   (E608, Sennheiser [b]), 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 [c]) 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 [d]), butorphanol (0.1 mg/kg IV, Butador, Chanelle [e]), and procaine benzyl penicillin

107   (12 mg/kg IM, Depocillin, MSD animal health [f]).   Animals were then sedated with romifidine

108   (0.08 mg/kg IV, Sedivet, Boehringer Ingleheim Vetmedica [g]).   Xylazine (0.4 mg/kg IV,

109   Virbaxyl, Virbac [h]) 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[i]) 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 [j] 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.*[5]. 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   it's 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[k]). 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[k])

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[6] was recorded.   Additionally, for

141   each video, the left arytenoid cartilage was graded as stable, mildly unstably or markedly

142   unstable[7] 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[8] using

148   image analysis software (Image J[l]). 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[m]. 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 Protective Coding analysis (see S1), with the search for formant peaks

167   constrained to the following previously described[9] 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 x

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≤ 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[1]. 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[k]). 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).

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 $1/3^{rd}$ 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[2,4]. This finding is rather surprising, given that all grade

281 4 horses can be assumed to have grade C laryngeal function[10,11], 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[3], 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)[4]. 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[2-4,9], 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*[9]. In contrast to Derksen *et al.*'s[9] 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[2,3] 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   [8]. Although some surgeons must have suspected this for some time, because they routinely

332   advocate bilateral VeC[2,12], it is still common to only remove the left vocal fold in horses with

333   RLN[13-15]. The current study and that of Leutton and Lumsden[8] 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 [16]. 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[15], 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- [8] and post-

347 laryngoplasty [8,17,18]. 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 [8,17,18] 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.*[3].

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[2-4]. 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[2,3,9], which would all be expected to have grade C laryngeal function at

378   exercise[10,11], 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[19]. 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[2,3,4]. 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   [a] Videomed GmbH , Munich, Germany

438   [b] Sennheiser UK Ltd., Marlow, UK

439   [c] TEAC UK Ltd, Guildford, UK

440   [d] Norbrook, Corby, UK

441   [e] Chanelle UK, Hungerford, UK

442   [f] MSD animal health, Milton Keynes, UK

443   [g] Boehringer Ingelheim Vetmedica, Bracknell, UK

444   [h] Virbac, Bury St Edmunds, UK

445   [i] Jørgen Kruuse A/S, Langeskov, Denmark

446   [j] Karl storz GmbH & Co. KG, Tuttlingen, Germany

447   [k] Dechra Veterinary Products, Shewsbury, UK

448   [L] National Institutes of Health, Bethesda, Maryland, USA.

449   [m] MathWorks, Massachusetts, USA

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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.