Crib Biting and Equine Gastric Ulceration Syndrome: do horses that display oral stereotypies have altered gastric anatomy and physiology?

Daniels, S.P[a*] , Scott, L[a]., De Lavis[a], I, Linekar[a], A. and. Hemmings, A, J[a].

[a] School of Equine Management and Science, Royal Agricultural University, Cirencester, GL7 6JS

[*] Corresponding author simon.daniels@rau.ac.uk

Abstract

Equine Gastric Ulceration Syndrome (EGUS) and Crib biting are two separate conditions suffered by horses. Previous research has hypothesised causal relationships between these two conditions, whereby the behavior is driven by a requirement to stimulate saliva production to buffer gastric juice. However to date there is limited empirical evidence to support this notion. To identify if the anatomy and physiology of the equid stomach differed in crib biting (CB) horses and non-crib biting controls (N-CB) a two part experiment was conducted using cadaver stomachs. Twenty four stomachs (n=12) CB and (n=12) N-CB were collected from an abattoir. Duplicate 1.5 cm squared sections were taken from the fundic and pyloric mucosa for histology and H&E staining to identify gastrin (G) producing cells. Slides were scored using an adapted four point scale. A further 18 stomachs, (n=9) CB and (n=9) N-CB were collected to test the pH of the mucosa and digesta from the fundic and pyloric regions. G cell concentrations were analysed by Mann Whitney U-test. Stomach content pH was analysed by one-way ANOVA and L.S.D post *hoc.* Relationships between digesta and mucosal pH were evaluated by correlation. In both parts of the study there was no difference between the G-cell concentration (*P*>0.05) and pH (*P*>0.05) between CB and N-CB horses. There was a positive correlation between digesta and the mucosal surface of pyloric region in CB horses (R^2 0.66, *P*<0.001), but not in N-CB horses. These findings suggest, from cadavers, that CB and N-CB stomachs are not anatomically nor physiologically different. It is plausible that there is no direct inherent link between CB and EGUS rather that both conditions are linked to environmental and physiological stress.

Introduction

Crib biting (CB) is an oral stereotypical behaviour, whereby the animal seizes a solid

object in the incisor teeth, flexes the strap muscles of the neck and emits a clearly

audible grunting sound (McBride and Hemmings 2009). Horses that perform oral

stereotypies have altered neurological anatomy and physiology associated with

dopamine function (McBride and Hemmings, 2005). Recent evidence of neurological

changes in crib-biting horses also suggests that there is a stress coping dimension to

crib-biting behaviour (Hemmings *et al.,* 2018).

Equine Gastric Ulceration syndrome (EGUS) is highly prevalent within performance

horses (Sykes *et al.,* 2015) but it has also been identified in horses in light work,

broodmares and semi-feral animals (Le Jeune *et al.,* 2005; Chameroy *et al.*, 2006;

Ward *et al.,* 2015). However, domesticated horses in work appear to suffer EGUS

with greater severity than semi-feral animals, suggesting a management role in the

syndrome (Ward *et al.,* 2015).

Previously CB and EGUS were linked in a group of crib biting foals that

demonstrated gastric lesions (Nichol *et al.,* 2002). From this work it was

hypothesized that crib biting horses have greater mucosal damage than non-crib

biting horses (Wickens *et al.,* 2013). The link between the two conditions is also in

the ECEIM consensus statement on EGUS (Sykes *et al.,* 2015). Oral stereotypy

(CB) has been postulated as a form self-medication, producing saliva, which buffers

the stomach pH (Hemmings and McBride, 2009).

Moller *et al.* (2008) concluded that crib biting horses produce less saliva than non-

CB controls, therefore perform the behaviour to produce saliva to buffer gastric juice.

This topic has recently been reviewed by Roberts *et al.* (2017) identifying several

studies that have proposed a link between CB and EGUS. However Houpt (2012)

83 directly measured saliva production in CB and non-CB horses found no difference in

84 the saliva production and concluded that CB did not stimulate saliva production and

85 was more likely to lead to ulcer formation by stimulation of gastric secretions.

86 Wickens *et al.* (2013) reported that in mature CB horses fed concentrates that there

87 was an increase in post prandial plasma gastrin levels compared to non-CB controls.

88 However no differences were observed in EGUS prevalence between CB and non-

89 CB horses (Wickens *et al.*, 2013). The findings of these previous studies suggests

90 that there may be a link between CB and EGUS, however the exact mechanism

91 linking both conditions is unclear.

92 The aim of this study was twofold, (1) to characterise concentrations of gastrin

93 producing cells in CB and N-CB cadaver stomachs and (2) measure the pH of the

94 fundic and pyloric mucosa and digesta in CB and N-CB cadaver stomachs.

95 **Methods**

96 Cadavers were sourced from horses that were slaughtered in the UK for human

97 consumption in continental Europe. The study met the ethical guidelines of the Royal

98 Agricultural University.

99 *Anatomy pilot study*

100 Six (n=6) mixed breed horses were identified at an abattoir in the South West of

101 England. Cadaver stomachs were collected directly following slaughter and

102 transported back to the laboratory in an insulated container. Tissue samples of 1.5 x

103 1.5 cm samples were taken in duplicate from the fundic and pyloric regions from

104 each stomach. Tissue was sectioned for histology and stained using H&E stain to

105 differentiate cells in each region of the stomach. Three slides per sample per region

106 were made (n=72). Slides were individually scored on a 1-4 scale adapted from

107   Creutzfeldt *et al.* (1976), in which 4 represents the highest concentration of G-cells,

108   (Figure 1). The pilot informed the quantification of G-Cells and confirmed the

109   expected differences in G-Cell concentrations between the fundic and pyloric regions

110   in N-CB horses.

111   *G-Cell quantification*

112   Twenty-four stomachs, 12 CB and 12 N-CB were used for G-cell

113   quantification. Cadavers came from animals aged from 3-22, mean 10 ± 7.62 years,

114   16 mares and 6 geldings, breeds comprised Thoroughbreds, Irish Sport Horses and

115   UK Native breeds. Crib biting was defined by observing the oral stereotypic

116   behaviour in lairage prior to slaughter. Post slaughter dental pathology, namely

117   oblique wear to the central incisors, was also used to confirm these animals were

118   crib biting outside of the abattoir environment. Stomachs were collected post

119   slaughter and 1.5 x 1.5 cm samples taken in duplicate from the fundic and pyloric

120   regions. Samples were processed histologically using H&E staining. G-cell

121   concentration was quantified using the 1-4 scoring method described previously.

122   *pH measurements*

123   Eighteen further cadaver stomachs, CB (n=9) N-CB (n=9) were collected for pH

124   measurements. CB behaviour was identified as described for G-cell quantification.

125   Animals had a mean age of 17 ± 5 years, some horses ages were not documented

126   but estimated from dentition. Cadavers represented mares, (n=9) and geldings

127   (n=9), breeds were Thoroughbreds, sport horses or unrecorded but of sport horse

128   type.

129   Stomachs were collected post slaughter, part of the selection criteria were stomachs

130   with limited distention, this was determined by palpation of the stomach, to prevent

131   too much acid buffering from the forage content. Stomachs were transported to the

132   laboratory in an insulated box. On return stomachs were placed in an incubator at

133   37°C to ensure the temperature of the content would represent a live horse and

134   reflect a true pH. Incisions were made in the fundic and pyloric regions in duplicate

135   and pH of the lining was measured in each region with a hand held pH probe (Hanna

136   pHep, HI98128). The contents, collected from the pyloric region, was passed through

137   a muslin bag and the pH of the acid was measured using the hand held pH probe.

138   Following this stomachs were opened by an incision along the greater curvature to

139   check for ulceration.

140   *Data analysis*

141   For G-Cell quantification data were ordinal and analysed by Mann-Whitney U

142   test, the fundic and pyloric regions between CB ad N-CB groups were considered

143   separately. The pH data were analysed by one-way ANOVA and L.S.D post *hoc.*

144   Relationships between mucosal pH and digesta pH for both groups were analysed

145   by Pearson correlation. All statistics were carried out using Genstat 18th edition.

146   **Results**

147   From the pilot study it was confirmed that in N-CB horses there was a difference in

148   the concentration of G-cells between the fundic and pyloric regions (Z -2.6264,

149   p=0.007). In both the G-cell quantification and pH parts of the study non-glandular

150   ulceration was observed in both CB and N-CB horses.

151   When quantifying G-cells there was no difference between G cell concentrations in

152   the fundic region (P>0.05) or in the pyloric region (P>0.05) between CB and N-

153   CB horses, (see figure 2). There was no difference in pH between tissue regions or

154   pyloric digesta between CB and N-CB horses (P=0.9) (see Table 1).

155   There was a positive relationship overall between the pH of the digesta samples in

156   the stomach and the mucosal surface of the pyloric region R^2 0.5 (P<0.001) and also

157   a relationship between pH of digesta and the mucosal surface of the fundic region R^2

158   0.4 (P=0.002). When evaluated within CB and non-CB stomachs there was a

159   positive relationship between the pH of CB digesta and the mucosal surface of the

160   pyloric region R^2 0.66 (P<0.001), but no relationship with the fundic mucosal surface.

161   Nor were there significant relationships with the N-CB digesta pH and mucosal

162   surfaces of the fundic or pyloric regions.

163   **Discussion**

164   The findings of this study suggest that in cadaver stomachs of crib biting and non-

165   crib biting horses there was no difference anatomically in gastrin cell concentration.

166   Furthermore there was no difference in the pH of the digesta or the mucosal

167   surfaces of the fundic or pyloric regions between the two groups. In both parts of the

168   study gastric ulcers were observed in both CB and N-CB stomachs. Interestingly

169   overall there was a relationship between digesta pH and mucosal surface of both the

170   fundic and pyloric regions, but when this was evaluated within the treatment groups

171   only the digesta and pyloric mucosal surface pH correlated in CB cadavers but not in

172   N-CB cadavers.

173   From the outset it is important to understand the limitations to the study in that using

174   cadavers may not accurately reflect the physiology of the living stomach. However

175   this non-invasive approach has given further insight into the proposed link between

176   crib biting and EGUS. The concept that crib biting behaviour is partially driven by the

177   desire to ameliorate gastric discomfort is prevalent within the literature (Moeller *et al.,*

178   2008; McBride and Hemmings, 2009; Wickens and Heleski, 2010; Roberts *et al.,*

179 2017) and also prevalent with horse managers in practice. However the findings of

180 Houpt (2012) dispute this in that the mechanism of crib biting does not stimulate

181 saliva production. It is more likely that the behaviour would stimulate the vagus

182 nerve and increase HCl production within the stomach. In turn this would increase

183 gastrin secretion further increasing gastric acid secretion, however this was outside

184 the scope of this study to measure. The findings of our study suggest that crib biting

185 horses were not anatomically nor physiologically different than N-CB controls within

186 these samples, questioning the conclusions of these previous studies. If there is a

187 link between EGUS and CB it is more likely that crib biting activates neural pathways

188 to stimulate gastric secretion.

189 The only previous study to identify a link between crib biting and gastric activity was

190 Wickens *et al.* (2013) who reported an increase in post prandial plasma gastrin

191 following consumption of concentrate feeds. In Wickens *et al.* (2013) study while

192 there was a relationship between post gastrin levels and crib biting horses, whether

193 horses were crib biters or not there was no difference in EGUS prevalence between

194 CB and N-CB animals. It is important to remember that in the horse gastrin is not a

195 stress hormone, gastrin is secreted due to the presence of feed (Frape, 2010). It is

196 also noteworthy that G-Cells only play part of the role of HCL secretion into the equid

197 stomach. Gastrin is the hormone which stimulates secretion of gastric acid from

198 parietal cells present in both the fundic and pyloric regions (Frape, 2010). Gastrin

199 was previously identified to differ in CB horses when compared to non-CB horses

200 (Wickens *et al.*, 2013) hence G-cell quantification was the focus of this study.

201 Parietal cells were not quantified as part of the present study. This could be

202 investigated within CB and N-CB cadaver stomachs using a similar study design.

203 Within the ECEIM consensus statement on EGUS the potential link between CB and

204 EGUS is highlighted as unclear (Sykes et al., 2015). From all of the evidence

205 available to date it is entirely possible that there is no direct link between the two

206 conditions. Crib biting behaviour is proposed to be a mechanism of coping with

207 environmental stress (McBride and Hemmings, 2009). This is supported by data

208 derived from dopamine (McBride and Hemmings, 2005) and opioid (Hemmings *et al,*

209 2018) receptor studies along with non-invasive probes of dopamine transmission

210 including spontaneous eye blink rate and behavioural initiation rate (Roberts *et al.*,

211 2015). Similarly alongside environmental stressors recent findings by Omidi *et al.*

212 (2017) suggest that crib biting horses are also under cellular stress/oxidative stress

213 or reduced antioxidant function. Furthermore Omidi *et al.* (2018) identified that crib

214 biting horses have altered serum selenium concentrations especially after crib biting

215 episodes suggesting a reduced antioxidant function.

216 Similarly previous works have linked EGUS to environmental stressors (Andrews

217 and Nadeau, 1999; Vatistas *et al.,* 1999; Andrews *et al.,* 2005; Hepburn, 2011). More

218 recently Ward *et al.* (2015) scored EGUS legions in domesticated and semi-feral

219 animals following slaughter. Both groups of animals displayed lesions in the non-

220 glandular region but the severity was greater in the domesticated animals. Thus

221 suggesting that management and environment play a role in the syndrome. When

222 looking at management factors for EGUS the use of stable mirrors has shown

223 reduced plasma gastrin levels inferring a reduction in stress when animals believe

224 they have a companion with them (Hepburn, 2011). This finding also questions if

225 gastrin may be a useful stress hormone in the horse? Glandular ulcers have also

226 been associated with stress identified through stress hormone responses (Malmkvist

227 *et al.,* 2012). The idea of stress playing a role in gastric ulceration was originally

228   identified in humans where peptic ulcer formation has been linked to stress

229   (Yoshitomi *et al.,* 1986). Furthermore oxidative stress has also been identified as

230   playing a role in the pathogenesis of gastric ulceration in humans (Bhattacharyya *et*

231   *al.,* 2014). In the horse the findings of Martinez Aranzales *et al.* (2014) identified that

232   the non-steroidal anti-inflammatory drug phenylbutazone given orally decreased

233   antioxidant capacity and increased oxidative stress to the gastric mucosa. Berger *et*

234   *al.* (2011) found that a dietary supplement containing both bicarbonate buffers and

235   antioxidants improved ulcer scores of Standardbred horses in training. These

236   findings collectively suggest that both environmental stress and oxidative stress play

237   a role in equine gastric ulceration syndrome.

238   When considered together, it is entirely possible that crib biting and equine gastric

239   ulceration syndrome are not directly linked. However both conditions are linked to

240   environmental and cellular stressors. While this hypothesis requires validation, it is

241   plausible that stress is the link between the two conditions. With these findings in

242   mind it is possible that gastric ulcers are present in most horses but the severity

243   differs between animals and this may be influenced by stressors and differing

244   management regimens.

245   **Conclusion**

246   The findings of this study suggest that the stomachs from horses that crib bite are

247   not anatomically or physiologically different from stomachs of non-crib biting controls.

248   It is also entirely possible that the two conditions are not directly linked and instead

249   are separate clinical sequalae of environmental and cellular stress.

250   **Acknowledgements**

251 A short abstract from the G-Cell quantification was presented at the 12th Colic

252 Research Symposium, Kentucky July 2017. A separate short abstract on the pH data

253 was presented at the British Society of Animal Science Conference, Dublin April

254 2018. The study was funded by the Royal Agricultural University.

255 **Ethics** This study was given ethical approval by the RAU Ethics Committee.

256 **Authorship**

257 The experiments were designed by Simon Daniels and Andrew Hemmings. The

258 experiments were performed by Imogen de Lavis, Louise Scott, Annebel Linekar and

259 Simon Daniels. The data were analysed by Simon Daniels. The paper was written by

260 Simon Daniels and Andrew Hemmings.

261 **References**

262 Andrews, F. M. and Nadeau, J. A., 1999. Clinical syndromes of gastric ulceration in foals

263 and mature horses. Eq. Vet. J., Suppl 29, 30-33.

264 Andrews, F. M., Buchanan, B. R., Elliot, S. B., Clairday, N. A. and Edwards, L. H., 2005.

265 Gastric ulcers in horses. J. .Anim. Sci., 83, 18-21.

266 Berger, S., Schmidt, C. and van den Hoven, R., 2011. The effect of Acid protect® on ulcer

267 healing in Standardbred horses in active training. Pferdeheilkunde., 27, 26-30.

268 Bhattacharyya, A., Chattopadhyay, R., Mitra, S. and Crowe, S. E., 2014. Oxidative stress:

269 An essential factor in the pathogenesis of gastrointestinal mucosal diseases. Phys. Rev., 94,

270 329-354.

271 Chameroy, K. A., Nadeau, J. A., Bushmich, S. L., Dinger, J. E., Hoagland, T. A. and Saxton,

272 A. M., 2006. Prevalence of non-glandular gastric ulcers in horses involved in a university

273 riding programme. J. Eq. Vet. Sci., 26, 207-211.

274 Creutzfeldt, W., Arnold, R., Creutzfeldt, C. and Track, N. S., 1976. Mucosal gastrin

275 concentration, molecular forms of gastrin, number and ultrastructure of G-cells in patients

276 with duodenal ulcer. Gut., 17(10), 745-754.

277 Frape, D., 2010. Equine Nutrition and Feeding. 4th Ed, pp 5. Wiley-Blackwell, UK.

278 Hemmings, A., Hale, C. E., Parker, M. and McBride, S. D., 2018. Causal and functional

279 interpretation of mu and delta opioid receptor profiles in mesoaccumbens and nigrostriatal

280 pathways of an oral stereotypy phenotype. Behav. Brain. Res., (open access). 353, 108-113.

281 Hepburn, R., 2011. Gastric ulceration in horses. In Practice., 33, 116-124.

282 Houpt, K. A., 2012. A preliminary answer to the question of whether cribbing causes salivary

283 secretion. J. Vet. Behav., 7, 322-324.

284 Le Jeune, S. S., Nieto, J. E., Dechant, J. E. and Snyder, J. R., 2005. Prevalence of gastric

285 ulcers in Thoroughbred broodmares in pasture: A preliminary report. T. Vet. J., 181, 251-

286 255.

287 Malmkvist, J., Poulsen, J. M., Luthersson, N., Palme, R., Christensen, J. W. and

288 Sondergaard, E., 2012. Behaviour and stress response in horses with gastric ulceration.

289 Appl. Anim. Behav. Sci., 142, 160-167.

290 Martinez Aranzales, J. R., Candido de Andrade, B. S. and Silveria Alves, G. E., 2014. Orally

291 administered phenylbutazone causes oxidative stress in equine gastric mucosa. Journal of

292 Vet. Pharma. and Therapeutics., 38, 257-264.

293 McBride, S. D. and Hemmings, A, J., 2005. Altered mesoaccumbens and nigrostriatal

294 dopamine physiology is associated with stereotypy development in a non-rodent species.

295 Behav. Brain Res., 159, 113-118.

296 McBride, S. D. and Hemmings, A. J., 2009. A neurological perspective of equine stereotypy.

297 J. Eq. Vet. Sci., 29, 10-16.

298   Moller, B. A., McCall, C. A., Silverman, S. J. and McElhenney, W. H., 2008. Estimation of

299   saliva production in crib-biting and normal horses. J. Eq. Vet. Sci., 28, 85-90.

300   Nichol, C. J., Davidson, H. P. D., Harris, P. A., Walters, A. J. and Wilson, A. D., 2002. Study

301   of crib-biting and gastric inflammation and ulceration in young horses. Vet. Rec., 151, 658-

302   662.

303   Omidi, A., Vakili, S., Nazifi, S. and Parker, M. O., 2017. Acute-phase proteins, oxidative

304   stress, and antioxidant defense in crib-biting horses. J. Vet. Behav., 20, 31-36.

305   Omidi, A., Jafari, R., Nazifi, S. and Parker, M. O., 2018. Potential role for selenium in the

306   pathophysiology of crib-biting behaviour in horses. J. Vet. Behav., 23, 10-14.

307   Roberts, K., Hemmings, A. J., Moore-Colyer, M. and Hale, C., 2015. Cognitive differences in

308   horses performing locomotor versus oral stereotypic behaviour. App. Anim. Behav. Sci., 168,

309   37-44.

310   Roberts, K., Hemmings, A. J., McBride, S. D., Parker, M. O., 2017. Causal factors of oral

311   versus locomotor stereotypy in the horse. J. Vet. Behav., 20, 37-43.

312   Sykes, B. W., Hewetson, M., Hepburn, R. J., Luthersson, N. and Tamzali, Y., 2015.

313   European College of Internal Medicine Consensus Statement - Equine Gastric Ulcer

314   Syndrome in Adult Horses. J. Vet. Int. Med., 29, 1288-1299.

315   Vatistas, N. J., Sifferman, R. L., Holste, J., Cox, J. L., Pinalto, G. and Schultz, K. T., 1999.

316   Induction and maintenance of gastric ulceration in horses in simulated race training. Eq. Vet.

317   J., Suppl. 29, 40-44.

318   Ward, S., Sykes, B. W., Brown, H., Bishop, A. and Penaluna, L. A., 2015. A comparison of

319   the prevalence of gastric ulceration in feral and domesticated horses in the UK. Eq. Vet. Ed.,

320   27, 655-657.

321   Wickens, C. L. and Heleski, C. R., 2010. Crib-biting behaviour in horses: A review. App.

322   Anim. Behav. Sci., 128, 1-9.

323   Wickens, C. L., McCall, C. A., Bursian, S., Hanson, R., Heleski, C. R., Liesman, J. S. and

324   Trottier, N. L., 2013. Assesment of gastric ulceration and gastrin response in horses with a

325   history of crib-biting. J. Eq. Vet. Sci., 33, 739-745.

326   Yoshitomi, K., Maronpot, R. R., Solleveld, H. A., Boorman, G. A. and Eustis, S. L., 1986.

327   Forestomach ulcers in Crj:B6C3 (CS7BWhNCrj x C3H/HeNC'rj) FI mice. Lab. Anim. Sci., 36,

328   501-503.

329

330

Table 1. Mean pH of stomach regions and digesta for CB and non-CB stomachs, superscript letters which are the same denote values that are not significantly different ($P>0.05$).

CB Fundic	N-CB Fundic	CB Pyloric	N-CB Pyloric	CB Digesta	N-CB Digesta
4.822[a]	4.656[a]	4.592[a]	4.739[a]	4.489[a]	4.509[a]

333

334

Figure 1. Histological H&E stained section of gastric mucosa. This section was graded 4 on the 1-4 scale.

337

Figure 2. G cell distribution scoring for the pyloric and fundic ($P>0.05$) and pyloric ($P>0.05$) regions of CB and N- CB cadaver stomachs.