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

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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.

Keywords: Oral Stereotypies, Gastric Ulceration, Stress, Oxidative Stress, Stomach

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 behavour 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)

directly measured saliva production in CB and non-CB horses found no difference in the saliva production and concluded that CB did not stimulate saliva production and was more likely to lead to ulcer formation by stimulation of gastric secretions. Wickens *et al.* (2013) reported that in mature CB horses fed concentrates that there was an increase in post prandial plasma gastrin levels compared to non-CB controls. However no differences were observed in EGUS prevalence between CB and non-CB horses (Wickens *et al*., 2013). The findings of these previous studies suggests that there may be a link between CB and EGUS, however the exact mechanism linking both conditions is unclear.

The aim of this study was twofold, (1) to characterise concentrations of gastrin producing cells in CB and N-CB cadaver stomachs and (2) measure the pH of the fundic and pyloric mucosa and digesta in CB and N-CB cadaver stomachs.

Methods

Cadavers were sourced from horses that were slaughtered in the UK for human consumption in continental Europe. The study met the ethical guidelines of the Royal Agricultural University.

Anatomy pilot study

Six (n=6) mixed breed horses were identified at an abattoir in the South West of England. Cadaver stomachs were collected directly following slaughter and transported back to the laboratory in an insulated container. Tissue samples of 1.5 x 1.5 cm samples were taken in duplicate from the fundic and pyloric regions from each stomach. Tissue was sectioned for histology and stained using H&E stain to differentiate cells in each region of the stomach. Three slides per sample per region were made (n=72). Slides were individually scored on a 1-4 scale adapted from

Creutzfeldt *et al.* (1976), in which 4 represents the highest concentration of G-cells, (Figure 1). The pilot informed the quantification of G-Cells and confirmed the expected differences in G-Cell concentrations between the fundic and pyloric regions in N-CB horses.

G-Cell quantification

Twenty-four stomachs, 12 CB and 12 N-CB were used for G-cell quantification. Cadavers came from animals aged from 3-22, mean 10 ± 7.62 years, 16 mares and 6 geldings, breeds comprised Thoroughbreds, Irish Sport Horses and UK Native breeds. Crib biting was defined by observing the oral stereotypic behaviour in lairage prior to slaughter. Post slaughter dental pathology, namely oblique wear to the central incisors, was also used to confirm these animals were crib biting outside of the abattoir environment. Stomachs were collected post slaughter and 1.5 x 1.5 cm samples taken in duplicate from the fundic and pyloric regions. Samples were processed histologically using H&E staining. G-cell concentration was quantified using the 1-4 scoring method described previously.

pH measurements

Eighteen further cadaver stomachs, CB (n=9) N-CB (n=9) were collected for pH measurements. CB behaviour was identified as described for G-cell quantification. Animals had a mean age of 17 ± 5 years, some horses ages were not documented but estimated from dentition. Cadavers represented mares, (n=9) and geldings (n=9), breeds were Thoroughbreds, sport horses or unrecorded but of sport horse type.

Stomachs were collected post slaughter, part of the selection criteria were stomachs with limited distention, this was determined by palpation of the stomach, to prevent

too much acid buffering from the forage content. Stomachs were transported to the laboratory in an insulated box. On return stomachs were placed in an incubator at 37°C to ensure the temperature of the content would represent a live horse and reflect a true pH. Incisions were made in the fundic and pyloric regions in duplicate and pH of the lining was measured in each region with a hand held pH probe (Hanna pHep, HI98128). The contents, collected from the pyloric region, was passed through a muslin bag and the pH of the acid was measured using the hand held pH probe. Following this stomachs were opened by an incision along the greater curvature to check for ulceration.

Data analysis

For G-Cell quantification data were ordinal and analysed by Mann-Whitney U test, the fundic and pyloric regions between CB ad N-CB groups were considered separately. The pH data were analysed by one-way ANOVA and L.S.D post *hoc*. Relationships between mucosal pH and digesta pH for both groups were analysed by Pearson correlation. All statistics were carried out using Genstat 18th edition.

Results

From the pilot study it was confirmed that in N-CB horses there was a difference in the concentration of G-cells between the fundic and pyloric regions (Z -2.6264, p=0.007). In both the G-cell quantification and pH parts of the study non-glandular ulceration was observed in both CB and N-CB horses.

When quantifying G-cells there was no difference between G cell concentrations in the fundic region (P>0.05) or in the pyloric region (P>0.05) between CB and N-CB horses, (see figure 2). There was no difference in pH between tissue regions or pyloric digesta between CB and N-CB horses (P=0.9) (see Table 1).

There was a positive relationship overall between the pH of the digesta samples in the stomach and the mucosal surface of the pyloric region R^2 0.5 (P<0.001) and also a relationship between pH of digesta and the mucosal surface of the fundic region R^2 0.4 (P=0.002). When evaluated within CB and non-CB stomachs there was a positive relationship between the pH of CB digesta and the mucosal surface of the pyloric region R^2 0.66 (P<0.001), but no relationship with the fundic mucosal surface. Nor were there significant relationships with the N-CB digesta pH and mucosal surfaces of the fundic or pyloric regions.

Discussion

The findings of this study suggest that in cadaver stomachs of crib biting and non-crib biting horses there was no difference anatomically in gastrin cell concentration. Furthermore there was no difference in the pH of the digesta or the mucosal surfaces of the fundic or pyloric regions between the two groups. In both parts of the study gastric ulcers were observed in both CB and N-CB stomachs. Interestingly overall there was a relationship between digesta pH and mucosal surface of both the fundic and pyloric regions, but when this was evaluated within the treatment groups only the digesta and pyloric mucosal surface pH correlated in CB cadavers but not in N-CB cadavers.

From the outset it is important to understand the limitations to the study in that using cadavers may not accurately reflect the physiology of the living stomach. However this non-invasive approach has given further insight into the proposed link between crib biting and EGUS. The concept that crib biting behaviour is partially driven by the desire to ameliorate gastric discomfort is prevalent within the literature (Moeller *et al.,* 2008; McBride and Hemmings, 2009; Wickens and Heleski, 2010; Roberts *et al.,*

2017) and also prevalent with horse managers in practice. However the findings of Houpt (2012) dispute this in that the mechanism of crib biting does not stimulate saliva production. It is more likely that the behaviour would stimulate the vagus nerve and increase HCl production within the stomach. In turn this would increase gastrin secretion further increasing gastric acid secretion, however this was outside the scope of this study to measure. The findings of our study suggest that crib biting horses were not anatomically nor physiologically different than N-CB controls within these samples, questioning the conclusions of these previous studies. If there is a link between EGUS and CB it is more likely that crib biting activates neural pathways to stimulate gastric secretion.

The only previous study to identify a link between crib biting and gastric activity was Wickens *et al.* (2013) who reported an increase in post prandial plasma gastrin following consumption of concentrate feeds. In Wickens *et al.* (2013) study while there was a relationship between post gastrin levels and crib biting horses, whether horses were crib biters or not there was no difference in EGUS prevalence between CB and N-CB animals. It is important to remember that in the horse gastrin is not a stress hormone, gastrin is secreted due to the presence of feed (Frape, 2010). It is also noteworthy that G-Cells only play part of the role of HCL secretion into the equid stomach. Gastrin is the hormone which stimulates secretion of gastric acid from parietal cells present in both the fundic and pyloric regions (Frape, 2010). Gastrin was previously identified to differ in CB horses when compared to non-CB horses (Wickens *et al*., 2013) hence G-cell quantification was the focus of this study. Parietal cells were not quantified as part of the present study. This could be investigated within CB and N-CB cadaver stomachs using a similar study design.

Within the ECEIM consensus statement on EGUS the potential link between CB and EGUS is highlighted as unclear (Sykes et al., 2015). From all of the evidence available to date it is entirely possible that there is no direct link between the two conditions. Crib biting behaviour is proposed to be a mechanism of coping with environmental stress (McBride and Hemmings, 2009). This is supported by data derived from dopamine (McBride and Hemmings, 2005) and opioid (Hemmings *et al,* 2018) receptor studies along with non-invasive probes of dopamine transmission including spontaneous eye blink rate and behavioural initiation rate (Roberts *et al*., 2015). Similarly alongside environmental stressors recent findings by Omidi *et al.* (2017) suggest that crib biting horses are also under cellular stress/oxidative stress or reduced antioxidant function. Furthermore Omidi *et al.* (2018) identified that crib biting horses have altered serum selenium concentrations especially after crib biting episodes suggesting a reduced antioxidant function.

Similarly previous works have linked EGUS to environmental stressors (Andrews and Nadeau, 1999; Vatistas *et al.,* 1999; Andrews *et al.,* 2005; Hepburn, 2011). More recently Ward *et al.* (2015) scored EGUS legions in domesticated and semi-feral animals following slaughter. Both groups of animals displayed lesions in the non-glandular region but the severity was greater in the domesticated animals. Thus suggesting that management and environment play a role in the syndrome. When looking at management factors for EGUS the use of stable mirrors has shown reduced plasma gastrin levels inferring a reduction in stress when animals believe they have a companion with them (Hepburn, 2011). This finding also questions if gastrin may be a useful stress hormone in the horse? Glandular ulcers have also been associated with stress identified through stress hormone responses (Malmkvist *et al.,* 2012). The idea of stress playing a role in gastric ulceration was originally

identified in humans where peptic ulcer formation has been linked to stress (Yoshitomi *et al.,* 1986). Furthermore oxidative stress has also been identified as playing a role in the pathogenesis of gastric ulceration in humans (Bhattacharyya *et al.,* 2014). In the horse the findings of Martinez Aranzales *et al*. (2014) identified that the non-steroidal anti-inflammatory drug phenylbutazone given orally decreased antioxidant capacity and increased oxidative stress to the gastric mucosa. Berger *et al.* (2011) found that a dietary supplement containing both bicarbonate buffers and antioxidants improved ulcer scores of Standardbred horses in training. These findings collectively suggest that both environmental stress and oxidative stress play a role in equine gastric ulceration syndrome.

When considered together, it is entirely possible that crib biting and equine gastric ulceration syndrome are not directly linked. However both conditions are linked to environmental and cellular stressors. While this hypothesis requires validation, it is plausible that stress is the link between the two conditions. With these findings in mind it is possible that gastric ulcers are present in most horses but the severity differs between animals and this may be influenced by stressors and differing management regimens.

Conclusion

The findings of this study suggest that the stomachs from horses that crib bite are not anatomically or physiologically different from stomachs of non-crib biting controls. It is also entirely possible that the two conditions are not directly linked and instead are separate clinical sequalae of environmental and cellular stress.

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

Authorship

The experiments were designed by Simon Daniels and Andrew Hemmings. The experiments were performed by Imogen de Lavis, Louise Scott, Annebel Linekar and Simon Daniels. The data were analysed by Simon Daniels. The paper was written by Simon Daniels and Andrew Hemmings.

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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]

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

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.