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Crib biting and equine gastric ulceration syndrome: Do horses that display oral stereotypies have altered gastric anatomy and physiology?

Simon Paul Daniels*, Louise Scott, Imogen De Lavis, Annabel Linekar, Andrew James Hemmings

School of Equine Management and Science, Royal Agricultural University, Cirencester

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ABSTRACT

Equine gastric ulceration syndrome (EGUS) and crib biting (CB) are two separate conditions suffered by horses. Previous research has hypothesized 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 CB horses and non–crib-biting (N-CB) controls a two-part experiment was conducted using cadaver stomachs. Twenty-four stomachs (CB: n = 12 and N-CB: n = 12) 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, (CB: n = 9 and N-CB: n = 9) were collected to test the pH of the mucosa and digesta from the fundic and pyloric regions. G cell concentrations were analyzed by Mann Whitney U-test. Stomach content pH was analyzed 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 or 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 behavior, 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 CB horses also suggests that there is a dimension of attempted coping with stress to CB behavior (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 (Chameroy et al., 2006; Le Jeune et al., 2005; Ward et al., 2015). 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 CB foals that demonstrated gastric lesions (Nichol et al., 2002). From this work, it was hypothesized that CB horses have greater mucosal damage than non–crib-biting (N-CB) 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 (McBride and Hemmings, 2009).

* Address for reprint requests and correspondence: Daniels, S.P., School of Equine Management and Science, Royal Agricultural University, Stroud Road, Cirencester GL76JS, United Kingdom. Tel: +4401285889847.
E-mail address: simon.daniels@rau.ac.uk (S.P. Daniels).

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Moller et al. (2008) concluded that CB horses produce less saliva than N-CB controls, therefore, perform the behavior 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 N-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, there was an increase in postprandial plasma gastrin levels compared with N-CB controls. However, no differences were observed in EGUS prevalence between CB and N-CB horses (Wickens et al., 2013). The findings of these previous studies suggest 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 two-fold, (1) to characterize concentrations of gastrin-producing cells in CB and N-CB cadaver stomachs and (2) to 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 after slaughter and transported back to the laboratory in an insulated container. Tissue samples of 1.5 × 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.

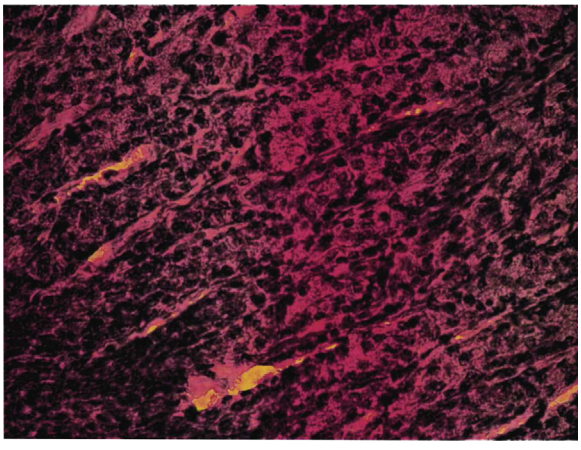

Figure 1. Histological H&E stained section of gastric mucosa. This section was graded 4 on the 1-4 scale.

G-cell quantification

Twenty-four stomachs, 12 CB and 12 N-CB, were used for G-cell quantification. Cadavers came from animals aged 3-22 years, mean 10 ± 7.62 years, 16 mares and 6 geldings; breeds comprised Thoroughbreds, Irish sport horses, and UK native breeds. CB was defined by observing the oral stereotypic behavior in lairage before slaughter. Postslaughter dental pathology, namely oblique wear to the central incisors, was also used to confirm if these animals were CB outside of the abattoir environment. Stomachs were collected after slaughter and 1.5 × 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 behavior 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 after slaughter, part of the selection criteria was 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, were passed through a muslin bag and the pH of the acid was measured using the hand-held pH probe. After 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 analyzed by Mann-Whitney U test; the fundic and pyloric regions between CB ad N-CB groups were considered separately. The pH data were analyzed by one-way ANOVA and L.S.D *post hoc*. Relationships between mucosal pH and digesta pH for both groups were analyzed by Pearson correlation. All statistics were carried out using Genstat 18th edition.

Results

This pilot study confirmed that N-CB horses differed in the concentration of G-cells between the fundic and pyloric regions (Z -2.6264, $P = 0.007$) with respect to both the G-cell quantification and pH parts.

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).

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 between pH of digesta and the mucosal surface of the fundic region (R^2 0.4; $P = 0.002$). 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

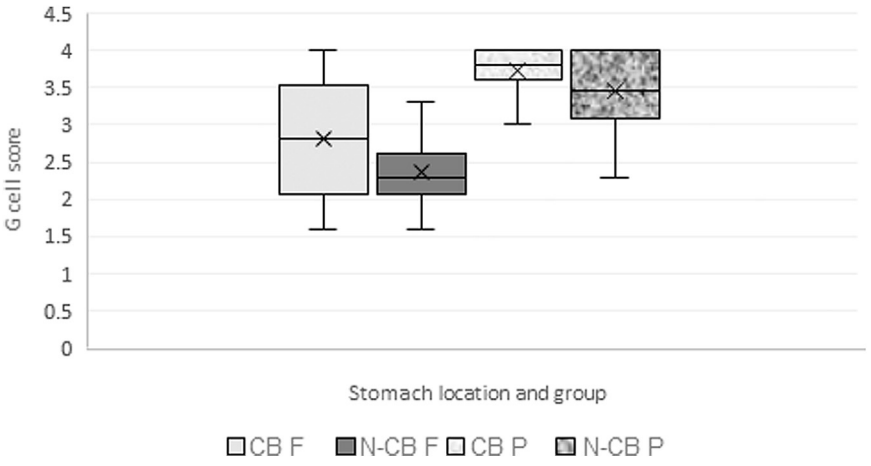

Figure 2. G-cell distribution scoring for the pyloric and fundic ($P > 0.05$) and pyloric ($P > 0.05$) regions of CB and N-CB cadaver stomachs.

with the fundic mucosal surface. There were no significant relationships with the N-CB digesta pH and mucosal surfaces of the fundic or pyloric regions.

Discussion

These results suggest that in cadaver stomachs of CB and N-CB horses there was no difference anatomically in gastrin cell concentration. Furthermore, there was no difference in the pH of the digesta, 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 parameter was evaluated within the treatment groups, only the digesta and pyloric mucosal surface pH correlated in CB cadavers. There was no correlative association in N-CB cadavers.

It is important to understand the limitations of the study. Use of cadavers may not accurately reflect the physiology of the living stomach. However, this noninvasive approach has given further insight into the proposed link between CB and EGUS. The concept that CB behavior is partially driven by the desire to ameliorate gastric discomfort is prevalent within the literature (Moller et al., 2008; McBride and Hemmings, 2009; Roberts et al., 2017; Wickens and Heleski, 2010) and with horse managers in practice. However, the findings of Houpt (2012) dispute this assertion because CB does not stimulate saliva production.

It is more likely that the behavior stimulates the vagus nerve and increases HCl production within the stomach. In turn, this could increase gastrin secretion further increasing gastric acid secretion; however, this hypothetical mechanism was outside the scope of this study to measure.

The findings of our study suggest that CB horses were not anatomically or 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 CB activates neural pathways to stimulate gastric secretion.

The only previous study to identify a link between CB and gastric activity was Wickens et al. (2013) who reported an increase in postprandial plasma gastrin after consumption of concentrate feeds. Wickens et al. (2013) found that while there was a relationship between post gastrin levels and CB horses, CB status had no effect on EGUS prevalence. It is important to remember that gastrin is not a stress hormone in the horse, but is secreted due to the presence of feed (Frape, 2010). 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 shown to differ in CB horses when compared with N-CB horses (Wickens et al., 2013), so G-cell quantification was the focus of this study. Parietal cells were not quantified as part of the present study. Parietal cell quantification 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. CB behavior is proposed to be a mechanism of coping with environmental stress (McBride and Hemmings, 2009). This supposition is supported by data derived from dopamine (McBride and Hemmings, 2005) and opioid (Hemmings et al., 2018) receptor studies, including those using noninvasive probes of dopamine transmission (spontaneous eye blink rate and behavioral initiation rate) (Roberts et al., 2015). Omidi et al. (2017) suggest that CB horses are also under cellular stress/oxidative stress or reduced antioxidant function. Furthermore, Omidi et al. (2018) demonstrated that CB horses have altered serum selenium concentrations after CB episodes, suggesting a reduced antioxidant function.

Earlier research has similarly linked EGUS to environmental stressors (Andrews and Nadeau, 1999; Andrews et al., 2005; Hepburn, 2011; Vatistas et al., 1999). More recently Ward et al. (2015) scored EGUS lesions in domesticated and semi-feral animals after slaughter. Both groups of animals displayed lesions in the nonglandular region of the stomach, but the severity of the lesions was greater in the domesticated animals. Accordingly, it has been suggested that management and environment play a role in the syndrome. Stable mirrors have been considered an intervention for improving management. Their use has shown reduced plasma gastrin levels, implying that there is a reduction in stress when horses 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 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 developed when peptic ulcer formation in humans was

Table
Mean pH of stomach regions and digesta for CB and N-CB stomachs

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]

Superscript letters which are the same denote values that are not significantly different ($P > 0.05$).

purported to be linked to environmental stress (Yoshitomi et al., 1986). Oxidative stress has also been identified as playing a role in the pathogenesis of gastric ulceration in humans (Bhattacharyya et al., 2014). Martinez Aranzales et al. (2014) found that the nonsteroidal anti-inflammatory drug phenylbutazone given orally to horses 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.

It is entirely possible that CB 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 N-CB 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.

Acknowledgments

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Authors' contributions: 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 analyzed by Simon Daniels. The article was written by Simon Daniels and Andrew Hemmings.

Ethical considerations

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

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