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Behaviour and stress responses in horses with gastric ulceration

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ABSTRACT

Only little is known about behaviour and stress responses in horses with gastric ulceration, despite the high prevalence of this condition. Our objectives in the present study was to (i) describe the severity of gastric ulceration in horses, housed under relatively standardised conditions, and (ii) to investigate whether horses with severe glandular gastric ulceration have increased baseline and response concentration of stress hormones and behave differently than control horses. We investigated stomachs of 96 horses at one stud, and compared an ulcer group ($n = 30$; with severe lesions in the glandular mucosa) to paired controls ($n = 30$; free from gastric ulcers). Baseline and response concentrations of faecal cortisol metabolites (FCM), heart rate and behaviour were measured in a novel object test (NOT, Day 1) and behaviour during postponed feeding (PF, Day 2). Glandular lesions occurred in 55.2% and non-glandular lesions in 40.6% of the horses. The amount of starch in the feed ($P = 0.006$) and paternal stallion ($P = 0.031$) influenced ulceration in the non-glandular region only; it should be noted that our study does not allow for separating hereditary from environmental influences, as offspring may be e.g. trained differently dependent on breeding line. Ulcer horses pawed more ($P < 0.001$) and ate quicker ($P = 0.050$) during PF. Although displayed by ulcers horses only during PF, we failed to demonstrate a significant association between glandular gastric ulceration and crib-biting/weaving; the total number of horses with these types of abnormal behaviour was low ($n = 5$). Behaviour and heart rate did not differ between groups in the NOT. Baseline concentration of FCM was similar ($P = 0.79$), however, ulcer horses responded stronger to novelty than controls (26% higher FCM; $P = 0.018$). We conclude that the prevalence of gastric ulcers was high, and our results suggest different factors affecting ulceration in the glandular versus the non-glandular region of the horse stomach. Obvious external signs (e.g. poor body condition) identifying ulcer horses were absent. Horses with severe glandular ulcers had a higher stress hormone response to novelty, thus they were more stress sensitive. Consequently, management evoking stress in horses should be reduced to dampen the development of glandular ulceration, or to protect horses with this condition.

1. Introduction

Gastric ulceration is frequently observed in horses in hard training – 86% of 345 not randomly selected race-horses (with performance problems in Begg and O'Sullivan, 2003), 93% of 30 high-level endurance horses during the competition season, reduced to 48% outside this season

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(Tamzali et al., 2011) – and in horses used for leisure riding (53% of 201 horses in Luthersson et al., 2009a).

Gastric ulceration occurs in both the non-glandular squamous and the glandular portion of the equine stomach, with squamous ulcers most frequently reported, but also the most studied (Bell et al., 2007a) and more easily accessed during gastroscopic examination (Murray et al., 2001). Intensity and duration of training/competition activity increases the occurrence of gastric ulceration in the squamous mucosa, presumably following increased exposure of this less protected part to acidic gastric contents during exercise (Tamzali et al., 2011). In the glandular part of the stomach a protective mucous-bicarbonate layer reduces the risk of acid injury (Bell et al., 2007a). Yet 33% of examined horses may have gastric lesions here (Tamzali et al., 2011). In classical experiments with laboratory rodents, glandular gastric ulceration has been related to stress (Selye, 1936; Weiss, 1968; Sapolsky, 2005). Similarly in horses, stress-induced release of endogenous cortisol has been proposed to increase the risk of developing gastric ulceration; e.g. by reducing the regenerating capacity of the glandular mucosa leading to less resistance towards ulceration (Andrews et al., 2005), of which the horse may be particularly prone due to a constant secretion of gastric acid according to their natural foraging behaviour with continuous grazing. *Helicobactor* spp. has not been found associated with stomach lesions in horses (Husted et al., 2010).

In the present study, we describe gastric ulceration in 96 sport horses kept under relatively similar conditions, at one commercial stud. Whereas a few studies have focussed on risk factors and the pathogenesis of equine gastric ulceration (e.g. Andrews et al., 2005; Bell et al., 2007b; Nadeau and Andrews, 2009; Luthersson et al., 2009b), the consequences for the horse are less well studied. It is unknown whether severely affected horses are in a state of stress or respond differently to acute stressors, which is of importance for both horse performance and welfare. We aimed to investigate whether horses with severe glandular gastric ulceration have higher baseline concentrations of stress hormones, and react differently (1) in a novel object test, and (2) during postponed feeding. During tests, we additionally studied whether eating behaviour and the occurrence of abnormal behaviour – such as crib-biting previously linked to ulceration in young horses (Nicol et al., 2002) – differ between adult horses with and without severe glandular gastric ulcers.

2. Materials and methods

2.1. Animals

We used Danish warmblood horses at one stud ($n = 98$; 17 mares, 33 geldings, 48 stallions), aged between 3 and 19 years, bred for competition in dressage or jumping, and housed individually in boxes (ca. 3 m × 3 m) on straw bedding. Data collected for each horse were: age, gender, paternity, body weight, body condition (according to scale in Henneke et al., 1983), stable within stud (1–5), training status (defined as ‘in training’, if exercised by humans for at least 5 h weekly), type/number of daily meals, and the amount of starch per meal. The health was evaluated by two veterinarians prior to the endoscopic examination; no individuals were excluded due to signs of illness.

Table 1
Experimental groups of control ($n = 30$) and ulcer ($n = 30$) horses. Medians with 25%; 75% quartiles, and means with SE.

Item	Control horses	Ulcer horses
[a]Glandular ulcer score, median	1 [1; 1]	3 [3; 3]
Range	0–1	3–4
[a]Nonglandular ulcer score, median	1 [1; 1]	1 [1; 2]
Range	0–1	0–3
Age (years)	7 (0.7)	7 (0.4)
Gender (mare, gelding, stallion)	M:6, G:8, S:16	M:5, G:13, S:12
Body weight (kg)	530 (7.9)	532 (8.9)
Body condition score (median)	5 [4; 5]	5 [5; 5]
Proportion of horses in training (%)	67	77
Starch feed per meal (g/kg BW)	1.1 (0.06)	1.1 (0.09)
[b]Hay served per day (kg)	4.2 (0.83)	4.2 (0.86)

[a] Using the EGUS scoring system described in Andrews et al. (1999).
[b] Excluding two horses per group with ad libitum access.

The horses were examined using a flexible video endoscope of 300 cm according to the method described in (Luthersson et al., 2009a), including fastening for 16 h, deprived of water for 5 h and sedated with detomidine (10–15 μg/kg BW i.v., Domosedan vet, Orion Corporation, Espoo, Finland) prior to the endoscopic examination. Gastric ulceration was scored for (1) the oesophagal non-glandular squamous mucosa and (2) the glandular mucosa of the stomach, simultaneously by two veterinarians, followed by an agreement on the final scores for the horse. The lesion grading system for Equine Gastric Ulcer Syndrome (EGUS) recommended by the Equine Gastric Ulcer Council was used (Andrews et al., 1999), with EGUS score 0 representing a healthy mucosa and score 4 for extensive and deep lesions. Two horses could not be assessed satisfactorily due to feed residuals in the stomach.

The experiment comply with the ‘Principles of animal care’, publication no. 86-23, revised 1985 of the National Institute of Health, and with current Danish laws. Informed owner consent was given. The owner, trainers and stable managers were blind regarding the ulceration scores, until after the experimental period; then veterinary advice regarding treatment was provided.

2.2. Experimental procedure

We selected horses to two groups: an ulcer group ($n = 30$) with lesions in the gastric glandular mucosa, EGUS scores 3–4, and a control group with intact mucosa (EGUS scores 0–1) (Table 1). EGUS grade 1 and below represents an intact mucosa, thus these horses have no ulceration present, but may have signs of reddening or hyperkeratosis (Nadeau and Andrews, 2009). Thirty pairs were made by pairing similar (regarding age, gender, body weight, paternity) horses. Each pair (one ulcer and one control horse) were tested on the same test days between 1 and 2.5 weeks after the gastroscopic examination, alternating between the ulcer and the control horse as the first to be tested. Test pairs were randomly distributed to two consecutive test days, with two pairs initiated per test day. Horses were tested in a novel object test (NOT, Day 1: 09:00–13:00 h), and exposed to postponed feeding (Day 2: 07:00–08:00 h). The observers were blind to the groups (ulcer, control) during the data collection.

2.3. Novel object test (NOT)

Novel object tests are used in several species to determine approach and avoidance reactions indicative of exploration and fear (e.g. Malmkvist and Hansen, 2002). Likewise, reactions towards novel objects have been quantified and related to fearfulness in horses (see work by Christensen et al., 2008, 2011, 2012; Christensen, 2012). In the present set-up, we test the horses' reactions to the novel object presented in their home environment, to avoid surplus handling of the animals and avoid the influence of social isolation/need of habituation, which would be necessary if a test arena was used. Our experimental horses were older and thus more experienced than the horses in previous novel object tests (e.g. 1–3 year-old horses; references earlier mentioned). Therefore, we added movement to the novel object, to increase the level of stimulus intensity. The horse was fitted with heart rate (HR) equipment (Polar Equine RS800, Polar Electro OY, Kempele, Finland) in their home box, followed by 3 min for habituation before testing. The novel object consisted of a traffic cone (height 50 cm, orange with two white stripes) fitted with a motor-driven 15 cm rod capable of moving up and down (1 time/sec) from inside the top of the cone when activated. The novel object was placed close to the front box wall equidistant from the two side walls. All horses were tested in their home box for 10 min (for the first 30 s with movement and sound from the motor), during which behaviour (Table 2) were recorded continuously on a handheld computer (Workabout, Psion PLC, London, UK) by an observer standing quietly 1 m outside the box.

2.4. Postponed feeding (PF)

On test Day 2, the feeding of horses to be tested was postponed with 1 h relative to their normal feeding time (starting daily at 07:00 h), whereas all other horses in the stable were fed as usual. Thirty minutes after the passing of the feeding trolley, the test horses were given approximately 15 g of their usual concentrate. Postponed feeding allows us to observe eating behaviour of horses under standardised conditions, after a fixed period of feed restriction and when they receive an equal amount of feed – a small fraction of their usual concentrate. Tests with small amounts of palatable feed have previously been used to evaluate cribbing in horses (Nagy et al., 2009). In addition, we obtain data on feeding motivation (latency to eat, eating duration). Behaviour (Table 2) during the 1 h (starting from the passing of the feeding trolley) of PF was analysed from recordings, obtained by digital cameras with build-in infrared lightning (Monacor TVCCD, Newport Pagnell, UK) above each box, using MSH-video software (M. Shafro & Co., Riga, Latvia).

2.5. Faecal cortisol metabolites (FCM)

FCM in horses reflect circulating blood cortisol with a 24 h time-lag (Möstl et al., 1999). For determination of baseline FCM, one sample of fresh faeces was collected non-invasively between 08:00 and 12:00 h, before the NOT. Determination of response FCM was done in faeces delivered 24 h (±30 min) after the NOT. Within 20 min after defecation, faeces were sampled from the bedding, homogenised and weighed (0.50 g) into vials. The samples were kept frozen until analysis.

Following extraction (Merl et al., 2000), 11,17-dioxoandrostanes (a group of FCMs) were determined by an 11-oxoaetiocholanolone enzyme immunoassay (Palme and Möstl, 1997), successfully validated for horses (Möstl et al., 1999). The inter- and intra assay coefficients were 9.5 and 11.3% respectively, and the assay sensitivity 0.9 ng/g faeces.

2.6. Data analysis

We used the procedure 'Mixed' in the SAS software (Statistical Analysis Systems Institute, Cary, USA), unless otherwise stated, with experimental group (ulcer, control) as fixed factor in models. Horse age (3–19 years), gender (mare, gelding, stallion), paternity (1–6), body weight (440–640 kg), body condition (4–6), stable (1–5), training status (0, 1), hay feeding (0, 1), and the amount of starch fed per meal (0–1.86 g/kg BW) were included as covariates/cofactors in the start models, as well as the interaction between the factors gender and group. Behaviour registered by counts was analysed in a similar generalized linear model using Poisson distribution (Procedure 'Genmod' in SAS; Dobson, 1997).

EGUS scores of the original stock ($n = 96$) were analysed as (1) score in a normal linear model and (2) as the probability of horses having ulcers (EGUS score of 2 or above) or not (scores 0–1) in a Binomial generalized linear model (Dobson, 1997) using the procedure 'Genmod' in SAS, including the same cofactors/covariates as described earlier.

The start models were reduced by stepwise removal of insignificant ($P > 0.1$) terms, keeping the main fixed factor in the final model. The requirement for dispersion and variance homogeneity was tested, and the validity of the final model judged by the appearance of the residuals, being improved for some variables after logarithmic or square-root transformation. In case of a significant class effect, pairwise comparisons were made using post-tests on least square means.

Analyses of latencies were done in SigmaPlot version 11.0 (Systat Software inc., Chicago, USA), using Kaplan–Meier survival analysis (log-rank method), thereby taking horses not reacting within the test time (censored data) into account (Kalbfleisch and Prentice, 2002).

A probability level (P) of 0.05 was chosen as a limit for statistical significance. Infrequent behaviour performed by less than 10% of the animals in the NOT: defecation (once

Table 2
Definitions of horse behaviour during the Novel Object (NOT) and Postponsed Feeding (PF) tests.

Behavioural variable	Definition
Standing (NOT/PF)	Standing relaxed
Walking (NOT/PF)	Walking energetically or relaxed
Alert (NOT/PF)	Elevated neck and head. In NOT distinguishing between orientations: (i) towards the novel object, (ii) in other directions than the novel object
Investigate (NOT)	Neck horizontal or lower, head and ears oriented towards (i) novel object or (ii) towards other directions than the novel object
Sniff novel object (NOT)	Head within 50 cm of the novel object, neck horizontal or lower, clear exhalations from nostrils
Touch novel object (NOT)	Touching or manipulating the novel object with mouth/muzzle
Stereotypic behaviour (NOT/PF)	A uniform pattern of movement apparently without purpose (e.g. pacing, weaving, head shaking) repeated minimum three times without interruption
Eat (NOT/PF)	Eating straw from the bedding or feed in the crib with visible chewing movements. In PF distinguishing between eating: (i) concentrate, (ii) hay/straw from the bedding
Lick (PF)	Licking or nibbling movements, divided into directed towards (i) crib area: mouth in contact with crib area or within 30 cm of the crib, no visible chewing, (ii) inventory: mouth in contact with water dispenser area or stable fittings
Drink (PF)	Mouth in contact with the water dispenser for more than 5 s
Paw[a] (NOT/PF)	One foreleg extended quickly forward, followed by movement backward, dragging the toe against the ground in a digging motion
Kick[a] (NOT)	The horse lift its weight on its forelegs and extend one or both hindlegs in a rapid motion
Rear[a] (NOT)	The horse rears more than 50 cm and bears its weight on the hindlegs
Snort[a] (NOT)	Powerful exhalation from the nostrils
Flehmen[a] (NOT)	Head elevated and neck extended and the upper lip everted, exposing the upper incisors and adjacent gums while drawing in air or fluids
Defecate[a] (NOT)	Elimination of faeces
Threat[a] (PF)	Laying ears flat back with or without turning in the direction of a neighbour
Kick abdomen[a] (PF)	Kicking in direction of the abdomen with one leg
Tail swishing[a] (PF)	Rapidly moving tail from side to side or up and down at least 45 degrees from normal vertical relaxed position
Crib-biting[a] (PF)	Horse latching onto a solid object with its incisors, arching its neck and pulling backwards
Tongue activity[a] (PF)	Extraneous moving of the tongue in and out of the mouth
Weaving[a] (PF)	Rhythmic, three times repeated side-to-side shifting of weight on the forelegs
Other (NOT/PF)	Other types of behaviour than above

[a] Only counts, no duration, recorded for this variable.

in 4 horses), flehmen (once in 3 horses), rearing (once in 2 horses) and kick, stereotypies (not occurring) – was not analysed further.

3. Results

3.1. EGUS scores in the original stock

The prevalence of horses with an EGUS scores of 2–4 (lesions in the mucosa) was 40.6% for the non-glandular squamous and 55.2% for the glandular portion of the stomach (Fig. 1). The median (25; 75% quartiles) score in the population ($n = 96$) was 1 (1; 2) for the non-glandular and 2 (1; 3) for the glandular part. One horse had unaffected epithelium (score 0), whereas 26 horses (27.1%) had score 2 and higher, in both examined regions. The correlation in EGUS score between the squamous and glandular region of the horses' stomach was low ($r_{spearman} = 0.17$; $P = 0.098$; Fig. 2).

Three stallions fathered the majority (89%) of the population (1: $n = 39$, 2: $n = 32$, and 3: $n = 14$), with no influence ($F_{2,80} = 1.0$, $P = 0.36$) on the glandular EGUS score in the offspring of these stallions; however, the EGUS score of the squamous region differed ($F_{2,71} = 3.7$, $P = 0.031$). Stallion 1 offspring had lower squamous EGUS score than Stallion 3 offspring (1.5 (0.30) vs. 2.2 (0.36); post-testing $P = 0.012$); offspring from Stallion 2 did not differ from the others (1.9 (0.26); post-testing $P > 0.070$). Furthermore, the EGUS score of the squamous region – but not of the glandular region – increased with the amount of starch fed per day ($F_{1,71} = 8.2$, $P = 0.006$; Fig. 3).

Analysing the data as binomial – considering the risk of having a gastric ulcer or not – also showed that the incidence of squamous ulceration depended on

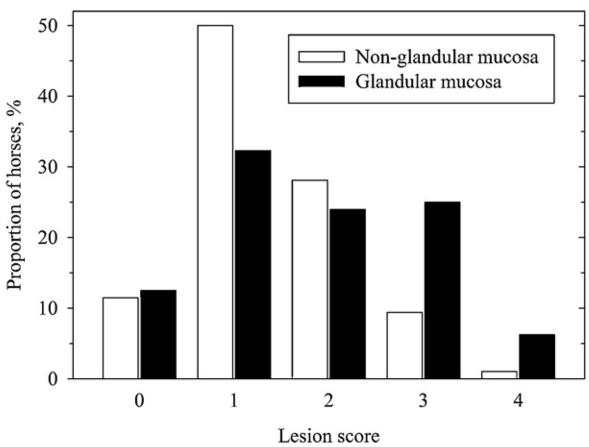

Fig. 1. Distribution of lesion scores in horses ($n = 96$). EGUS score 0: intact epithelium without reddening or hyperkeratosis, 1: intact mucosa with areas of reddening or hyperkeratosis, 2: small single or multifocal lesions, 3: large, single or multifocal lesions or extensive superficial lesions, and 4: extensive lesions with areas of apparent deep ulceration. Grading was done in the (1) squamous non-glandular mucosa in the upper half and (2) the glandular mucosa in the cardia, fundic and pyloric regions, in the lower part of the stomach.

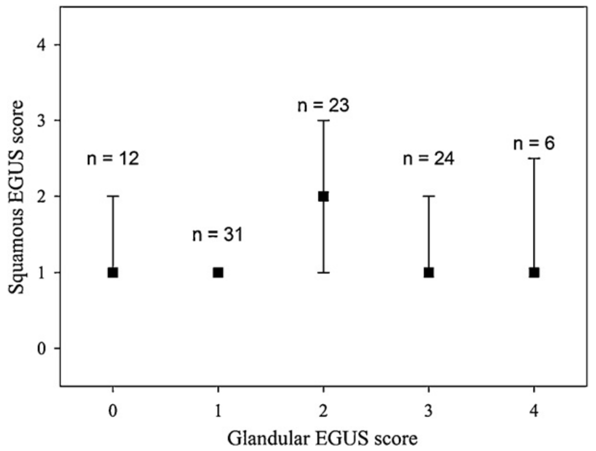

Fig. 2. EGUS score (median with 25; 75% quartiles) in the squamous mucosa for each of the EGUS scorings in the glandular mucosa of the horse stomach (n = 96). The correlation between scores in the two regions were low ($r_{spearman}$ = 0.17; P = 0.098).

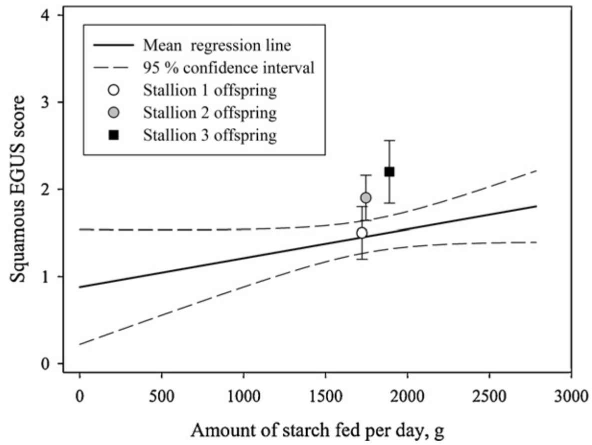

Fig. 3. EGUS score in the gastric squamous region modelled as mean regression line with 95% confidence intervals versus the amount of starch fed per day. The mean (s.e.) score for the offspring from three dominant paternal stallions is plotted. Both paternity (P = 0.031; post-test: S3 > S1) and starch in diet (P = 0.006) influenced the EGUS score in the squamous, but not in the glandular region.

paternity (χ^2 = 6.6, P = 0.037; post-test: Stallion 1 < Stallion 2, P = 0.029, odds-ratio (OR) = 0.17 (confidence limits: 0.11-0.92); Stallion 1 < Stallion 3, P = 0.029, OR = 0.23 (0.06–0.89); Stallion 2 vs. Stallion 3, P = 0.59).

3.2. Ulcer versus control horses

3.2.1. Baseline concentration of faecal cortisol metabolites (FCM)

Ulcer and control horses did not differ in their baseline concentration of FCM (ulcer: 5.7 (2.03) vs. control: 5.3 (1.93) ng/g, $F_{1,47}$ = 0.1, P = 0.79).

3.2.2. Novel object test

Ulcer horses tended to spend more time away from the novel object (P = 0.056), and they had on average a 26% higher concentration of FCM 24 h after the NOT ($F_{1,33}$ = 6.3, P = 0.018) compared to controls (Table 3). Time spent being alert towards the novel object differed between gender ($F_{1,44}$ = 5.3, P = 0.009), as mares spent less time alert (7.0%) than geldings (15.5%) and stallions (21.3%; post-tests P < 0.023).

Snorting occurred in 69.0% of the horses (1–12 times), but did not differ between groups ($ChSQ_{1,44}$ = 0.0, P = 0.84). Pawing occurred in 31.0% of the horses (1–23 times), and did not differ between groups ($ChSQ_{1,51}$ = 1.1, P = 0.29), but was influenced by gender ($ChSQ_{1,44}$ = 47.8, P < 0.001); geldings pawed less (average 0.4) than mares (2.1) and stallions (2.9) during the NOT (post-tests P < 0.001).

Table 3
Novel object test responses: latencies (median [25%; 75%]) and behaviour (% of observation time), heart rate during test, and FCM in horses with severe glandular ulceration and controls.

Variable	Control	Ulcer	Test statistics	P-value
Behaviour				
Latency, sniff novel object (s)	87 [52; 215]	82 [55; 239]	$LR_{df=1}$ = 0.0	0.90
Proportion censored (%)	18.8	19.2		
Latency, touch novel object (s)	100 [51; 262]	102 [56; 253]	$LR_{df=1}$ = 0.0	0.92
Proportion censored (%)	15.6	23.1		
Time spent away novel object (%)	35.4 (6.18)	46.8 (6.6)	$F_{1,50}$ = 3.8	0.056
Standing, (%)	17.4 (2.67)	16.9 (3.19)	$F_{1,44}$ = 0.5	0.56
Walking, %	1.6 (0.44)	1.0 (0.17)	$F_{1,49}$ = 1.3	0.27
Alert novel object (%)	5.6 (0.71)	4.9 (0.73)	$F_{1,50}$ = 0.3	0.56
Alert other (%)	2.2 (0.44)	3.1 (1.13)	$F_{1,49}$ = 1.5	0.23
Investigate novel object (%)	1.9 (0.52)	1.9 (0.40)	$F_{1,50}$ = 0.2	0.70
Investigate other (%)	3.0 (0.58)	4.1 (0.86)	$F_{1,45}$ = 0.3	0.58
Sniff novel object (%)	4.5 (0.71)	4.6 (0.85)	$F_{1,50}$ = 0.4	0.54
Touch novel object (%)	25.9 (4.48)	24.2 (4.86)	$F_{1,45}$ = 0.6	0.43
Eat (%)	23.7 (4.97)	22.8 (5.73)	$F_{1,44}$ = 0.0	0.99
Heart rate				
Average the first 2 min	45 (1.3)	43 (1.4)	$F_{1,51}$ = 0.7	0.42
Maximum	71 (3.3)	69 (3.7)	$F_{1,51}$ = 0.3	0.58
FCM				
Baseline concentration (ng/g)	5.3 (1.93)	5.7 (2.03)	$F_{1,47}$ = 0.1	0.79
Response concentration (ng/g)	5.8 (0.79)	7.3 (0.81)	$F_{1,33}$ = 6.3	0.018

Table 4
Observations during the 1 h postponed feeding test, as proportion of observation time (mean ± se, %) in horses with severe glandular ulcers and controls.

Variable	Control	Ulcer	Test statistics	*P*-value
Standing	18.4 (3.19)	21.4 (3.61)	$F_{1,36}=1.2$	0.28
Walking	4.2 (0.63)	3.1 (0.39)	$F_{1,40}=1.8$	0.19
Alert	6.8 (0.63)	3.1 (0.36)	$F_{1,35}=0.5$	0.48
Eating concentrate	4.3 (0.47)	3.5 (0.41)	$F_{1,38}=4.0$	0.050
Eating roughage	29.8 (4.75)	30.0 (6.07)	$F_{1,40}=0.0$	0.98
Licking	10.8 (2.47)	7.8 (1.82)	$F_{1,33}=0.8$	0.38
Drinking	0.2 (0.09)	0.5 (0.23)	$F_{1,21}=0.4$	0.44

3.2.3. Postponed feeding

Ulcer horses ate the small amount of concentrate 19% quicker than control horses ($F_{1,38}=4.0$, $P=0.050$, Table 4). Gender also influenced the eating duration ($F_{2,38}=5.8$, $P=0.006$), as geldings ate slower (3.1 min) than mares (1.6 min, post-test $P=0.002$) and stallions (2.2 min, $P=0.023$).

Among ulcer horses, one showed crib-biting and four weaved, whereas none of the controls performed these behaviours. The low number of horses performing crib-biting and weaving rendered statistical analysis infeasible; behaviour counted in less than 15% of the horses were not analysed statistically, excluding counts of crib-biting (2.6% of horses), weaving (7.9% of horses) and social threats (14.3%, equal number of ulcer and control horses). Extraneous tongue activity (48.8% of horses) was displayed more often by control (10.8) than by ulcer horses (5.3 times) ($ChSQ_{1,30}=28.7$, $P<0.001$). Horses in training displayed 'tongue activity' 1.8 times more often than the rest ($ChSQ_{1,30}=15.3$, $P<0.001$), and the behaviour increased with the amount of starch fed per day ($ChSQ_{1,30}=8.3$, $P=0.004$). Among horses displaying abdominal kicks and tail swishing ($n=19$), the amount of kicks towards the abdomen (range 1–14) and tail swishing (range 1–175) were positively correlated ($r_{pearson}=0.62$, $P=0.004$), indicating that these types of behaviour may be linked to the same state, e.g. pain or frustration. Ulcer and control horses did not, however, differ in frequency of kicks to abdomen ($ChSQ_{1,31}=2.4$, $P=0.12$) nor in tail swishing ($ChSQ_{1,30}=0.6$, $P=0.46$). Ulcer horsed pawed more than control horses (6 [2; 17] vs. 2 [0; 14] times, $ChSQ_{1,30}=102.8$, $P<0.001$) during the 1 h observation period. Additionally, the statistical model predicted an increase of 3 pawings/h per unit starch added to the feed per day ($P<0.001$).

4. Discussion

4.1. Gastric ulceration in riding horses

Investigating 96 horses at one stud, we confirm a high prevalence of gastric ulceration in riding horses used for dressage and jumping. The prevalence of horses with lesions in the mucosa (EGUS scores 2–4) was 40.6% for the squamous and 55.2% for the glandular portion of the stomach. Our data indicate a different nature behind ulceration in the two parts of the horse stomach; the correlation between ulceration in the two regions was low, and the amount of starch in the diet increased EGUS scores in the squamous region only (Fig. 3). Likewise, previous studies have demonstrated that feeding management – including the amount of starch (Luthersson et al., 2009b), limited hay feeding (McClure et al., 1999) and periods of feed deprivation (Murray and Eichorn, 1996) – is a risk factor for lesions in the squamous region, whereas this is not evident for the glandular ulcers. Our study supports the view of differentiation between gastric ulceration in the upper squamous part and deeper glandular part of the horse stomach. We were surprised to find more and worse lesions in the glandular (median EGUS score 2) than in the squamous region (median EGUS score 1). This contrasts findings in racehorses (Murray et al., 1996), where lesions in the gastric squamous epithelium scored higher. Tamzali et al. (2011) also recorded a higher prevalence for squamous (93%) than for glandular (33%) ulceration in endurance horses during competition (riding distance: 90–160 km). This discrepancy in severity and primary ulceration site could reflect the different types of feeding and training between studies. Our experimental horses were likely less exposed to intense/extended periods with acidic gastric content pushed up into the upper squamous region than is the case for the horses in racing and endurance riding activities.

We report a paternal influence (three stallions fathering 89%) on the risk of having gastric ulceration. It is well-known in other mammals that traits such as fearfulness and stress-sensitivity have a genetic component (e.g. Malmkvist and Hansen, 2002; Malmkvist et al., 2003). It should be recognized, however, that the reported paternal effect not only reflects hereditary influences, as offspring from stallion 1 and 2 primarily were used for dressage and stallion 3 offspring primarily for jumping, although not exclusively. Additionally, the difference in quality and usage of offspring is likely to be confounded with other differences in e.g. feeding and training intensity/competition level; factors which could be risk factors, but not included in great details in our data collection.

4.2. Differences between healthy horses and horses with severe glandular ulceration

A visual inspection by two experienced horse veterinarians did not reveal any obvious external signs of horses with severe ulceration. All horses were in good body condition (cf. Table 1), contrasting the suggestion that ulcer horses generally are thin or look shabby (cf. clinical signs mentioned in Begg and O'Sullivan, 2003).

Horses with glandular ulceration had in average a 26% higher cortisol concentration in response to novelty, whereas their baseline concentration did not differ from controls. Thus horses with severe glandular ulceration were more stress-sensitive. It has been suggested that stress-induced release of endogenous cortisol increases the risk of developing gastric ulceration; e.g. by reducing the regenerating capacity of the glandular mucosa leading to less resistance towards ulceration (Sapolsky, 2005; Andrews et al., 2005). From our study, we can not deduce whether the higher cortisol release following a standardised stressor caused horses to be more prone to glandular ulcers or whether the higher release is a consequence of having ulcers. Nevertheless, the present result links stress-sensitivity and glandular ulcers in horses. Thus management factors evoking stress should be carefully considered to reduce the development or to protect horses that already have developed glandular ulceration.

Despite the severity of the glandular lesions (EGUS scores 3–4) in ulcer horses, we failed to demonstrate them being in more pain – as for e.g. indicated by more kicking towards the abdomen, tail swishing, or interrupted eating – than controls. Neither did ulcer horses have a higher baseline concentration of faecal cortisol metabolites (FCM), in other studies linked to acute pain (e.g. during the days after castration and in horses with colic in Merl et al., 2000).

Crib-biting and other types of abnormal behaviour have been identified as a sign of frustration and/or stress in situations with arousal such as feeding time (Nagy et al., 2009). Studies suggest that crib-biting horses are more stress-sensitive than control horses (McGreevy and Nicol, 1998; Bachmann et al., 2003). In pigs, a weak association has been described between the frequency of redirected oral behaviour and the risk of acute ulcers in the fundus region of the stomach (Dybkjær et al., 1994). Likewise, crib-biting foals had a higher incidence of squamous gastric lesions (Nicol et al., 2002). In the present case-control study, we failed to demonstrate an association between the traditional land-marks of abnormal behaviour (crib-biting, weaving), excessive licking, and glandular ulceration in adult horses. Although displayed by ulcer horses only, few horses in total (five) displayed crib-biting and weaving; therefore the predictive value of using the occurrence of crib-biting and weaving to differentiate between horses free from ulcers and horses with severe glandular ulceration was close to zero.

Intuitively, we expected horses with severe glandular ulcers to eat slower, with interrupted eating bouts, due to abdominal discomfort. This was not the case. In fact, ulcer horses ate the delivered amount of feed quicker than control horses during the postponed feeding test. This indicates that ulcer horses are more eager to obtain food, which also could explain their triple amount of pawing, indicative of anticipation of food/frustration of not receiving food, during the postponed feeding test.

The reasoning behind the postponed feeding test was to observe eating behaviour of horses under standardised conditions, i.e. when receiving a small amount of concentrate after a period of restriction following feeding cues. Postponed feeding test is also used in farmed mink, to reveal stereotypic behaviour (Malmkvist et al., 2012), which can be latent and under influence of the feeding regime. Stereotypies in horses are predicted to occur mainly post-eating, however, could also be influenced by lack of satiation following eating and other factors as well (see Mason and Mendl, 1997 for discussion). Our personal observation is that crib-biting in affected horses frequently occurs during or immediately after ingestion of concentrates. Likewise, Nagy et al. (2009) report a feeding test with delivery of small amount of feed (tidbits) to induce cribbing in crib-biting horses.

During the postponed feeding test, control horses displayed about twice as much extraneous tongue activity than ulcer horses. We are not aware of the function behind this behaviour, displayed more in horses in training and increasing with the amount of daily starch allocation. Whether this type of tongue activity – decreased in horses with glandular ulcers – is linked to any particular motivation, a part of healthy eating behaviour or rather a type of abnormal behaviour awaits further studies.

5. Conclusion and perspectives

We report a high prevalence of gastric ulceration in riding horses used for dressage and jumping, with lesions (EGUS scores 2–4) in the glandular portion of the stomach present in 55.2% of the studied horses. The daily starch amount and the paternity of the horse were risk factors for ulcers in the squamous gastric region only. Our study material does not allow us to separate hereditary from environmental influences, as offspring may be e.g. trained differently dependent on breeding line.

Based on a case-control study, it was not possible to use external signs in e.g. body condition, or frequency of crib-biting/weaving as reliable indicators of severe glandular ulcers. Horses with severe glandular ulcers were more eager to eat when deprived, and had a higher stress hormone response to novelty compared to controls. Management factors evoking stress should therefore be carefully considered to dampen the development of severe glandular ulceration or to protect horses with this condition.

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