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Master's Thesis in Veterinary Medicine

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Evaluation of musculoskeletal pain and behavior during tacking-up and riding in horses with equine gastric ulcer syndrome

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Title: Evaluation of musculoskeletal pain and behavior during tacking-up and riding in horses with equine gastric ulcer syndrome

Topic description: An observational cohort study aimed to determine the relationship between musculoskeletal pain, behavior during tacking-up, and riding in horses diagnosed with equine gastric ulcer syndrome.

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Preface

This thesis was conducted as a final part of a Master's Thesis in veterinary medicine at the University of Copenhagen, Faculty of Health and Medical Sciences. The study undertaken from February 2024 to August 2024, focused on investigating behavior during tacking-up and ridden exercise in horses. Data for the study was collected from horses who were admitted for gastroscopy at Large Animal Teaching Hospital due to suspicion of equine gastric ulcer syndrome.

The findings of this study have relevance for veterinary students, veterinarians, and other professionals with an interest in EGUS, behavior, and musculoskeletal pain.

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Abbreviations

AAEP: American Association of Equine Practitioners

EGGD: Equine glandular gastric disease

EGUS: Equine gastric ulcer syndrome

ESGD: Equine squamous gastric disease

LATH: Large Animal Teaching Hospital

LL: Lameness locator

RHpE: Ridden Horse pain Ethogram

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1. Background

1.1 Equine gastric ulcer syndrome

1.1.1 Anatomical structures of the equine stomach

The equine stomach is relatively small compared to the size of the horse and has a capacity of five to fifteen liters depending on the size of the horse. The esophagus enters the stomach in the cardiac part. The sphincter muscle between the stomach and esophagus prevents the content from refluxing [1]. The stomach is divided into two parts, the non-glandular area, and the glandular area, separated by the margo plicatus. The non-glandular area of the stomach is covered with squamous cell epithelium and constitutes a third of the whole equine stomach, where pH is neutral [1]. The other two-thirds of the stomach is called the glandular area and consists of glandular tissue that produces gastric acid, pepsin, bicarbonate, and mucus [1]. The caudal part of the glandular area ends in the pyloric area, leading to the pylorus and continuing to the duodenum. The production of gastric acid does not depend on digestion or the amount of content in the stomach [2].

1.1.2 Definition

Equine gastric ulcer syndrome (EGUS) is used to describe both erosive and ulcerative mucosal diseases in the equine stomach. The syndrome was first described in 1999 [3] and was recently divided into equine squamous gastric disease (ESGD) and equine glandular gastric disease (EGGD), to provide a more specific description depending on the anatomic location. ESGD refers to changes in the squamous part of the stomach including the margo plicatus, the curvatures, and dorsal squamous fundus. EGGD describes changes in the glandular mucosa in the glandular part of the stomach including the cardia, ventral glandular antrum, the pyloric antrum, and the first part of the duodenum [4].

1.1.3 Prevalence

The prevalence of EGUS varies across different studies depending on breed and environmental differences. The prevalence of ESGD is well described and exhibits more variability in appearance compared to EGGD. A Danish study including 201 pleasure horses of different ages and breeds, primarily Warmblood horses, found a prevalence of ulcers with a severity grade ≥ 1 at 69% for ESGD and 57% for EGGD [5]. For racehorses, it has been demonstrated that during the interseason up to 58% exhibited ESGD and 17% EGGD, while during competition season, these numbers increased to 93% for ESGD and 33% for EGGD [6]. In contrast, a study including 80 university-owned horses showed a

prevalence of ESGD at 11%, these horses were rarely participating in competitions and mainly exercised within their home environment [7].

1.1.4 Etiology/pathophysiology

ESGD is initiated by increased exposure of acid to the mucosa, and studies have shown that a pH below 1.7 will have a detrimental effect on the epithelium [8]. Hydrochloric and volatile fatty acids lower the pH and participate in the development of squamous ulcers, depending on the time and the dose [9,10]. The erosion of the mucous membrane will extend into the spinous layer, creating a conducive environment for ulcers to form [9]. Splashing of gastric acid can occur when the horse is not eating enough roughage and therefore not getting enough fibers. Fibers will prolong the chewing time which will produce a bigger amount of saliva that provides a buffering effect in the stomach. The fibers will also create a ball of roughage in the stomach, which will decrease acid splashing [11]. Intensive training in trot and canter will cause contraction of the stomach, and the gastric acid will therefore be forced up into the proximal part, thereby being exposed to the non-glandular mucosa [12]. The predilection site for ulcerations in the non-glandular part is in the margo plicatus, especially in the greater curvature. Ulcerations are less often seen in the dorsal part [5]. ESGD occurs as a primary or a secondary form depending on the pathophysiology. The primary form is the most common and occurs in a healthy gastrointestinal tract, while the secondary form arises as a result of sequelae from other gastrointestinal diseases for example delayed gastric output and pyloric stenosis [4]. Damage to the glandular mucosal occurs when the protective mechanism of the mucosa is weakened, but it is not further described in horses. In humans, the two predominant factors are the bacteria *Helicobacter Pylori* and the use of NSAIDs [4]. The site of predilection of EGGD is in the pyloric region but can also be manifested in the cardiac and fundic regions [5]. The majority of the EGGD lesions are mainly found in the pyloric antrum [4].

1.1.5 Risk factors

Describing risk factors for developing EGUS is difficult due to the various factors that can influence its development. Studies have found that no access to water in the paddock, relying solely on straw as roughage, intervals of more than 6 hours between feedings, starch at a rate exceeding 2g/kg body weight per day or >1g/kg body weight per meal are risk factors for EGUS [13]. Allowing horses to graze freely on pasture year-round without monitoring their feed intake may pose a potential risk factor for the development of EGUS in Icelandic horses [14]. The intensity of training is also proved

to increase the risk of developing EGUS since the prevalence of both ESGD and EGGD is doubled in the competition season due to participating in a competitive environment compared to the inter-season in a study of endurance horses [6].

Equine squamous gastric disease

Various risk factors have been identified for ESGD including traveling of horses and not being turned out in a paddock [15]. Hay feeding less than three times per day increased the risk of ESGD development in both polo horses and Icelandic horses [14,15]. Studies have indicated that horses exhibit a circadian rhythm that increases rest and decreases consumption of roughage during the late-night hours [16], culminating in a low pH in the early morning. Consequently, engaging in exercise during the morning hours should be avoided, as it may increase the risk of exposing the stomach to gastric acid [17]. Furthermore, prolonged periods of training, exceeding six weeks, and display of all types of stereotypical behavior, is also shown as risk factors in Thoroughbreds [18]. In a study with Thoroughbred horses in training, they demonstrated no correlation between using NSAID and ESGD [19]. Exercising in trot or canter in total for more than 270 minutes per week will increase the risk 20 times of developing ESGD [15].

Equine glandular gastric disease

The risk factors for EGGD include engaging in exercising more than five times per week, the trainer, racing below the expected performance level [18], and not being turned out on pasture [15]. Furthermore, having multiple caretakers and riders around the horse may be stressful for some horses and are therefore all considered risk factors [20]. Foals experiencing stress exhibited a higher likelihood of developing gastric lesions, with 40% of the stressed foals displaying EGGD [21]. The use of phenylbutazone (NSAID) was found to elevate the risk of developing EGGD among a group of Quarter horses and Warmbloods [22]. In general, minimizing stress may decrease the risk of developing EGGD [18,23].

1.1.6 Clinical signs

Different clinical signs associated with EGUS have been described, e.g. colic, poor coat condition, reduced performance [19,24,25], diarrhea, decreased appetite [26], poor body condition [26,27], weight loss [24,25,28], girthing pain and abdominal pain [24,25] generally changes in rideability [25]

and behaviors [24,25,28], bruxism [25] and crib-biting [29]. Further, EGUS can be asymptomatically for some horses [26].

1.1.7 Diagnosis

The most reliable method to diagnose EGUS is gastroscopy. The examination requires an endoscope at least 2.5-3 m long, which can visualize the entire stomach, the antrum of the pylorus, and the proximal part of the duodenum. By using the endoscope, it is possible to identify all the different areas in the equine stomach and localize and grade the ulcers [4]. A study showed that endoscopy underestimated the number of ulcers and the depth and severity of ulcers, compared to necropsy and histological examinations [30]. The first to describe ESGD lesions with a scale was the Equine Gastric Ulcer Council [3], and this was later adapted by the European College of Equine Internal Medicine Consensus Statement to effectively classify the lesions with a grading system [4].

The scale, which ranges from 0-4 (Table 1), describes the severity of changes observed at the different stages of gastric ulcers [4]. Bleeding is not one of the markers in the ESGD grading system, because small superficial erosions can have active hemorrhage and deep ulcerations can occur without bleeding in a gastroscopy examination [3]. Back in 2015 the European College of Equine Internal Medicine Consensus Statement suggested not to use a scale to define EGGD lesions, but instead describe them by their anatomical location, distribution, severity, and appearance [4]. But later it is proven that an EGUS grading system has an acceptable inter- and intraobserver reliability for both ESGD and EGGD (Table 1) [31].

Table 1 | Equine gastric ulcer syndrome (EGUS) grading system differentiated into equine squamous gastric disease (ESGD) and equine glandular gastric disease (EGGD) [31].

Grade	ESGD	EGGD
0	The epithelium is intact and there is no appearance of hyperkeratosis	The epithelium is intact and there is no appearance of hyperemia
1	The mucosa is intact, but there are areas of hyperkeratosis	The epithelium is intact, but there are areas of hyperemia
2	Small, single, or multifocal lesions	Small, single, or multifocal lesions
3	Large, single, or extensive superficial lesions	Large, single, or extensive superficial lesions
4	Extensive lesions with areas of apparent deep ulceration	Extensive lesions with areas of apparent deep ulceration

1.1.8 Treatment

The most frequently used treatment for EGUS is the proton pump inhibitor, which is a type of medicine that decreases acid production by blocking the hydrogen-potassium-ATPase. This enzyme catalyzes the last step in the hydrochloric acid production in the parietal cells [32]. Omeprazole has become the recommended proton pump inhibitor to treat EGUS [4].

It is demonstrated that spontaneous healing of ESGD rarely occurs if no management and environmental changes are performed [14,33]. Murray et al. [33] demonstrated that horses diagnosed with EGUS and treated with omeprazole had faster healing of gastric ulcers than non-treated, and all treated ulcers were healed within 10-21 days. Multiple studies have shown that the treatment with omeprazole has a lower success rate for EGGD compared to ESGD. Complete healing of ESGD is 80-86% compared to 14-21% for EGGD [34,35]. The treatment of EGGD is not yet well understood because the pathophysiology is not fully understood either. However, the effectiveness of omeprazole will be enhanced if the treatment for EGGD is complemented by mucosal protection therapy with a medication such as sucralfate. Combination therapy should be administered for at least 8 weeks before considering alternative treatment [4].

It is recommended, that ESGD is treated for at least 3 weeks because studies showed that if the lesions heal, they will heal within 3 weeks [33]. The omeprazole treatment dose is recommended at 4.0 mg/kg once daily, but different studies have shown a good response to treatment with doses down to 1.0 mg/kg once daily [4,34,35]. It is recommended to administer oral omeprazole to the horses after fasting optimally 8 hours before treatment [36]. To facilitate management and due to the horse's circadian, omeprazole should be administered early in the morning, as horses normally consume less feed during the night and therefore can be fasting overnight without affecting the gastric pH very much [17]. In addition, to get the most optimal effect of omeprazole, the horse should not eat anything for a minimum of 30 minutes after the administration, and optimally 60-90 minutes after administration [36].

1.1.9 Preventing

Management has a major impact in preventing EGUS by avoiding several risk factors.

Horses should not have periods without roughage for longer than 6 hours [13]. Ad libitum feeding is a good choice for avoiding periods without roughage, but it is important to confirm that the horses eat enough [11]. For thrifty horses or horses who need a prolonged chewing time with less energy, it is possible to replace up to 50% of the roughage portion with straw, without increasing the risk of gastric ulcers [37]. Hay nets can be used to prolong the eating time [16]. Feeding with alfalfa hay can

decrease the risk of developing ESGD because it has a buffering effect on the gastric acid, and results in a higher gastric pH [38]. To prevent potential damage of the squamous mucosa caused by exposure to gastric acid, horses should avoid exercise on an empty stomach and preferably consume a minimum of 0.3 kg of hay for a maximum of 4 hours period before exercise, because it will increase the pH in the stomach and achieve the buffering effect [17].

There is limited evidence that diet has a great impact on preventing EGGD [4], but it is proven that if the horses exercise 5 or more times per week, the risk of EGGD increases. Therefore, it is recommended that horses have at least two full days or more free of all work [18,39]. In addition to incorporating rest days, facilitating cohabitation with other horses allows for the expression of natural behaviors, which may contribute to a reduction in stress levels and potentially minimize the risk of developing gastric ulcers [11].

1.2 Behavior

In the study of equine behavior, it is essential to distinguish between normal and abnormal behavior, as it is important to understand and recognize behavioral signs that may indicate that the horse is not comfortable. Abnormal behavior can be expressed in various situations, such as during handling, interactions with other horses, during tacking-up, mounting, and while ridden.

1.2.1 Behavior during riding

Ridden Horse pain Ethogram

The Ridden Horse pain Ethogram (RHpE) is an ethogram describing musculoskeletal pain in the ridden horse and was developed by Dr. Sue Dyson and Jeannine Berger [40]. It was inspired by earlier studies illustrating pain evaluated from facial expressions in stabled and ridden horses, and studies describing behavior during riding such as bucking and rearing associated with musculoskeletal pain [40]. The RHpE aimed to raise awareness about lameness in horses, as owner undetected lameness has a notable prevalence in ridden horses. Previous studies have indicated that approximately 38-75% of horses presumed to be sound by their owners, were lame [41,42]. Further, there is a general belief in the equestrian population that abnormal behavior during riding can appear from bad rider skills, inadequate education, and improper training methods rather than originating from musculoskeletal pain [43]. Based on this the RHpE was developed as a tool for owners, riders, and trainers to be more skilled in detecting signs of pain-related behavior in horses [40]. At the initial stages of the development of the RHpE, a total of 117 potential behavioral markers, were considered before limiting down

to the current 24 behavioral markers included in the ethogram (Table 2). This selection process was based on the analysis of numerous video recordings capturing lame and sound (non-lame) horses. The horses were ridden in trot in straight lines and 10 m circles and in canter, on both reins [40]. When applying the RHpE, the ridden horse should be observed for 5-10 minutes. The RHpE is designed for dressage horses or at least horses who can perform dressage-like exercises in an arena. It has not yet been developed for racehorses, western horses, or jumping/eventing horses [43], but a Danish study showed an adaptation of the RHpE to Icelandic horses including tölt as a gait [44]. As a basis for the scoring, the horse must be observed in walk, trot, and canter, in a riding arena on both reins. Performing exercises viewed from the perspective of the front, from both sides and behind, in straight lines, transitions, and 10 m circles on both reins performing consistently as figures of eight in rising trot [43]. A lame horse is at higher risk of displaying some of the 24 behavioral markers when compared to a sound horse, with a likelihood of more than 10 times. The presence of 8 or more of the 24 included behavioral markers in a ridden horse is indicative of musculoskeletal pain [40]. It is shown that when comparing the scores from 6 to 9, the threshold of 8 gave the best preferences with a specificity at 0.8 and a sensitivity at 0.86 [45]. A previous study applied the RHpE and collected data from competitions at various levels in dressage and eventing, where they found that several lame horses were participating. This group of lame horses exhibited significantly more of the 24 behavioral markers compared to the group of non-lame horses [46]. For truthful scoring of ridden horses with the RHpE the accessor must receive training in the correct use of the ethogram before the assessment

Table 2 | Equine ridden pain behavior – Final ethogram of 24 behavioral markers for scoring in ridden horses [39]

Ridden Horse Pain Ethogram (RHpE)	
1	Repeated changes of head position (up/down)
2	Head tilted or tilting repeatedly
3	Head in front of vertical (>30º) for ≥10s
4	Head behind vertical for ≥10s
5	Head position changes regularly, tossed or twisted from side to side, corrected constantly
6	Ears rotated back behind vertical or flat (both or one only) ≥5s; repeatedly lay flat
7	Eyelids closed or half closed for 2-5s
8	Sclera (white of eye) exposed
9	Intense stare for 5s
10	Mouth opening ± shutting repeatedly with separation of teeth, for ≥10s
11	Tongue exposed, protruding or hanging out and/or moving in and out
12	Bit pulled through the mouth on one side (left or right)
13	Tail clamped tightly to middle or held to one side
14	Tail swishing large movements: repeatedly up and down/side to side/circular; during transitions
15	A rushed gait (frequency of trot steps >40/15s); irregular rhythm in trot or canter; repeated changes of speed in trot or canter
16	Gait too slow (frequency of trot steps <35/15s); passage-like trot
17	Hindlimbs do not follow tracks of forelimbs but deviated to left or right; on 3 tracks in trot or canter
18	Canter repeated leg changes: repeated strike off wrong leg; change of leg in front and/or behind; disunited
19	Spontaneous changes of gait (e.g., breaks from canter to trot to canter)
20	Stumbles or trips/catches toe repeatedly
21	Sudden change of direction, against rider direction; spooking
22	Reluctance to move forward (has to be kicked ± verbal encouragement), stops spontaneously
23	Rearing (both forelimbs off the ground)
24	Bucking or kicking backwards (one or both hindlimbs)

[47]. When using the RHpE it is essential to agree that several scores must be present for a certain time or multiple times before it counts [40].

Before determining a specific behavioral marker for a given horse (Table 2), it is important to evaluate the horse and the tacking equipment. Some horses can have a small iris and therefore the sclera may be visible either unilaterally or bilateral at rest and when the horse is ridden and for that reason, that marker cannot be counted in the total score. Also, it is important to notice if the bit fits the horse's mouth, if the bit is too wide for the horse it could give a false impression when analyzing the "bit pulled through", and if the horse has a pink muzzle, it can be difficult to distinguish if the mouth is open or not. Likewise, the surface the horse is riding at should be noticed because it can impact the behavioral marker where the horse is evaluated with toe drag [43].

It is noteworthy that very heavy riders can impact the RHpE score, resulting in a higher total score when compared to riders with a lower body weight riding the same horses [48]. Opposite, it has been shown that the level of the rider does not impact the total RHpE score. While a skilled rider may be better to maintain the horse in balance and enhance the gait qualities, the RHpE scores will not improve [49].

1.2.2 Behavior during tacking-up

Causes

A protocol for behavior during tacking-up has been developed based on a study investigating the behavior of 193 horses during tacking-up, including bridling, saddling, and tightening of the girth. The study concluded that abnormal behavior during tacking-up including chomping at the bid, ears back, intense stare, biting, and tail swishing are common problems and may be related to stress and pain [50]. These behaviors can occur from tack-associated discomforts such as ill-fitting equipment, musculoskeletal pain, and lameness [51], oral lesions, or pain from riding [50]. Girthiness is an important clinical sign that may hint at an underlying condition such as gastric ulceration [52,53] or orthopedic problems [51,53]. Girthiness is often reported as an indication of gastric ulceration [53], and omeprazole treatment was found to reduce the abnormal behavior during tacking-up [50,53]. Although it is well known that several horses with gastric ulcers do not show any abnormal behaviors [54].

1.3 Musculoskeletal pain

1.3.1 Lameness

Lameness is a prevalent health issue among equines, manifesting across various countries and disciplines, such as racing and riding school horses [55–58]. Studies dating back to 1985 have underscored the substantial impact of lameness both in terms of prevalence [56] and the financial burden it places on horse owners [57]. Lameness represents a pervasive issue of reduced performance in horses, and several studies have highlighted the prevalence of musculoskeletal disorders encountered by veterinarians [55,56,58]. Lameness is, unfortunately, a more unrecognized problem for horses in training than suspected. In a study including riders, owners, and trainers that expected their horses to be sound, found that only 29.8-54.3% were concluded to be sound by veterinarians when undergoing a lameness examination [41,42].

1.3.2 Lameness examination

Subjective lameness examination

A lameness examination optimally consists of collecting full history, a clinical examination including palpation, inspection, and observing the horse in hand on a straight line at walk and trot, lunging on soft and hard surfaces on both reins, performing flexion tests, and observing the horse ridden, if possible. The aim of this examination is, to identify lameness in one or several limbs [59–61]. The process of lunging induces asymmetric movement in horses. When a horse is trotting in a circle, its vertical movement is typically affected symmetrically, when comparing the left circle to the right. An example of the effect of the circle is a more noticeable impact on the inner hindlimb. It is advisable to lunge the horse in both directions to ensure a balanced and accurate assessment of its gait. [62]. It is important to consider that certain horses may not exhibit lameness when evaluated solely in specific segments of the lameness examination. This fact highlights the limitations of relying only on observing the horse trotting in straight lines when assessing lameness [42].

Further diagnostic investigations to localize the lameness origin includes the use of diagnostic analgesia [61]. Once the affected area has been localized, diagnostic imaging techniques can provide further support and confirm the specific underlying disease responsible for the lameness [59,63].

To enhance the objectivity of subjective lameness assessment and to have the ability to describe the lameness in consistency and severity, both to other veterinarians and for documentation purposes, a scale is used for this purpose. For a very long period, lameness has primarily been graded worldwide by the American Association of Equine Practitioners (AAEP), on a scale from 0-5 (Table 3) [61]. Other scales exist to describe lameness, e.g. the United Kingdom Lameness Scale, which ranges from 0-10 [61]. In both scales grade 0 describes a non-lame horse in any circumstances. Dr. Sue Dyson has developed a lameness scale, that ranges from 0-8, and is categorized as sound (0), mild (2), moderate (4), severe (6), and nonweightbearing (8). This scale is described as more workable because it encompasses a broader range of gait abnormalities both during walking and trotting [64].

Table 3 | The American Association of Equine Practitioners (AAEP) lameness grading system is used to define the grade of lameness in this project.

AEEP lameness grading system	
Grade	**Description**
0	Lameness not perceptible under any circumstances.
1	Lameness is difficult to observe and is not consistently apparent, regardless of circumstances (e.g. under saddle, circling, inclines, hard surface, etc.)
2	Lameness is difficult to observe at a walk or when trotting in a straight line but consistently apparent under certain circumstances (e.g. weight-carrying, circling, inclines, hard surface, etc.)
3	Lameness is consistently observable at a trot under all circumstances.
4	Lameness is obvious at walk.
5	Lameness produces minimal weight bearing in motion and/or at rest or a complete inability to move.

Performing a subjective lameness examination can be supplemented by objective measurements to optimize accuracy. It is well known fact that the veterinarian's experience, and method of picking up lameness when watching a horse will influence the results. Therefore, a subjective lameness examination on the same horse can be evaluated and graded differently depending on which veterinarian performs the examination [65,66]. In general, veterinarians have a strong agreement when deciding if the horse is lame, but the agreement is lower when deciding which limb the horse is lame on after a full lameness examination [67]. Also, there is a stronger agreement recognizing forelimb lameness compared to hindlimb lameness [67,68]. When grading the lameness using the AAEP lameness score, the veterinarians mostly agree whenever the lameness represented a mean of >1.5. Therefore when evaluating a low-grade lameness a subjective lameness examination benefits greatly by supplementation of an objective lameness examination [67,69].

Objective lameness examination

The objective lameness examination is based on different types of technology systems, which aim to collect objective data, analyze the information, and thus detect vertical asymmetry in the horse [70].

These systems often involve the use of sensors placed in multiple locations on the horse, such as the pelvis, head, withers, and one or more limbs [70]. Additionally, some systems can evaluate the horse by recording its movement pattern using a mobile device [71].

The Equinosis Q with Lameness Locator® (LL) used in this project, consists of a sensor-based wireless system connected to a software system with the ability, to analyze the asymmetry in vertical acceleration in the trotting horse [72]. The system has an inertial measurement unit and once the horse is equipped with sensors and the LL is connected, data can be collected on the horse's movement [70]. The LL can gather data during trotting in straight lines and lunging on various types of surfaces, such as concrete, soft, and packed dirt. The three sensors are positioned on on the dorsum of the pelvis, the head, and the right forelimb pastern [61]. During a lameness examination with a supplement of an objective evaluation, the LL may guide the veterinarian with a detailed analysis of the horse's movement pattern [61]. Comparing the subjective and objective lameness examination prevents overlooking subtle lameness that may be relevant to the well-being of the horse. The disadvantage of such a sensitive system is that small possibly irrelevant asymmetries can be recognized. This leaves the veterinarian with the important task of recognizing clinically relevant asymmetries and lameness in the process of diagnosing the patient [73].

1.3.3 Back pain

A clinical examination of the back is a subjective assessment and should consist of inspection and palpating to check for swellings, atrophy, muscle fasciculations, muscle tension, muscle spasms, focal pain, etc. [74,75]. The horses should also be evaluated in movement, similar to a lameness examination, which includes walking and trotting up in straight lines and lunging in trot and canter with multiple transitions [61]. Where the most common signs of back pain will be observed as altered movement patterns, including stiffness and poor hindlimb impulsion [74].

Furthermore, testing the horse in flexing the entire spine in various directions, including ventroflexion, dorsal extension, and lateral bending on both sides and testing the ability to turn the neck up and down. If the horse is reluctant to flex, it may indicate back pain [61,75]. Other behaviors that may indicate back pain during palpation include dropping down when pressure is applied, tail swishing, kicking, biting, rotating the ears back, or vocalizing [74,75]. Furthermore, behavioral changes during riding, such as refusal to jump, rearing, bucking, difficulties with transitions, hindlimb collapse, and resistance during tacking-up should be noted [75].

When assessing the horse for back pain, it is also recommended to assess the saddle. Horses experiencing back pain may exhibit sensitivity in the withers and saddle area. Abnormal hair wear or white hair in the saddle region could indicate the onset of back pain resulting from ill-fitting saddles [41,75,76].

Soft tissue injuries and vertebral lesions are the most significant causes of back pain in horses. Specific diagnoses such as Dorsal spinous process impingement, muscular damage/ligamentous strains, and sacroiliac strain are the most common diagnoses in cases of back pain [77]. Dorsal spinous process impingement also known as “kissing spine” was already described in 1980 as the most common back disorder, conducted in a study based on 443 horses with suspected back disorders [77]. Recent studies indicate that as many as 39% of horses may have kissing spine, though not all cases result in clinical symptoms. However, horses with kissing spine are found to have a threefold increased risk of experiencing back pain [78].

Summary

Background: Musculoskeletal pain, equine gastric ulcer syndrome (EGUS), and abnormal behavior during tacking-up and riding are unfortunately very common in riding horses but have not yet been evaluated in the same study.

Objectives: To explore if EGUS is triggered by musculoskeletal pain evaluated by repeated Ridden Horse pain Ethogram (RHpE) scores, behavioral scores during tacking-up, as well as both an objective lameness examination and a subjective musculoskeletal evaluation in a group of horses diagnosed with EGUS, as well as after an EGUS treatment period.

Study Design: Observational cohort study.

Methods: The study included 44 horses at the Large Animal Teaching Hospital (LATH) at the University of Copenhagen, Denmark. The horses underwent an initial examination including a questionnaire, an equipment check, a recording of an objective lameness examination, a tacking-up, and a standardized riding program, followed by a gastroscopy the next day. The horses were included in the control examination if they were diagnosed with EGUS. The control examination contained a recording of an objective lameness examination, a tacking-up, and the same standardized riding program. The following day a subjective lameness examination, and a gastroscopy. The recordings of each horse were evaluated blinded and scored for tacking-up and RHpE scored for the standardized riding program. Descriptive statistics were employed for most of the data due to the few included horses. Data were compared using a Welch t-test, a Fisher's exact test, a simple t-test, and a paired t-test. The statistical agreements were calculated using bar charts, Bland-Altman plot, and Cohen's Kappa. The statistical significance was defined as $p<0.05$.

Results: A significant increase with 1-2 scores was found in the RHpE score in the group of horses with non-healing EGUS from the initial examination to the control examination ($p = 0.04$). Bland-Altman showed overall good agreement between observers. Cohen's Kappa showed actual agreement ranging from 59.4-96.9%, and strength of agreement ranging from poor to almost perfect.

Main limitations: Limited number of horses participating in the project.

Conclusion and potential relevance: The group of horses with non-healing EGUS, and with an increase in the RHpE score from the initial to control examination were all diagnosed with EGGD. Although horses with a RHpE score ≥8 and co-existing EGUS, displayed abnormal behavior, a significant correlation could not be confirmed due to the low sample size.

Keywords: Horse, musculoskeletal pain, ridden behavior, tacking-up behavior, gastric ulceration.

Resumé

Baggrund: Muskuloskeletal smerte, mavesår og unormal adfærd under opsadling og ridning er desværre meget hyppigt hos rideheste, men har indtil videre ikke været evalueret i det samme studie.

Formål: At undersøge om mavesår er påvirket af tilstedeværelsen af muskuloskeletal smerte, evalueret med Ridden Horse pain Ethogram (RHpE) score, adfærdsscoring ved opsadling, samt både en objektiv halthedsundersøgelse og en subjektiv muskuloskeletal evaluering, i en gruppe af heste diagnosticeret med mavesår og efter en periode med behandling for mavesår.

Studiedesign: Observerende kohorte studie.

Metode: Studiet inkluderede 44 heste på Universitetshospitalet for Store Husdyr i Danmark. Hestene gennemgik en initiel undersøgelse, som inkluderede et spørgeskema, et udstyrstjek, en videooptagelse af en objektiv halthedsundersøgelse, opsadling og et standardiseret rideprogram, samt en gastroskopi den efterfølgende dag. Hestene var inkluderet i kontrolundersøgelsen, hvis de blev diagnosticeret med mavesår. Kontrolundersøgelsen bestod af en videooptagelse af en objektiv halthedsundersøgelse, opsadling og det samme standardiserede rideprogram. Dagen efter blev der foretaget en subjektiv muskuloskeletal evaluering og en gastroskopi. Videooptagelserne af hver hest blev anonymiseret med nummer og scoret for opsadling og RHpE scoret for det standardiserede rideprogram. Deskreptiv statistik blev anvendt for det meste data, grundet få inkluderede heste. Data blev brugt til sammenligning med en Welch t-test, en Fisher's exact test, en simpel t-test og en parret t-test. Den statistiske enighed blev analyseret ved brug af søjlediagrammer, Bland-Altman plot og Cohen's Kappa. Det statistiske signifikansniveau blev sat til $p<0,05$.

Resultater: En signifikant stigning på 1-2 scorer blev fundet i RHpE scoren for gruppen af heste med ikke-helende mavesår mellem den initielle undersøgelse og kontrolundersøgelsen ($p<0,05$). Bland-Altman plottet viste en overvejende god enighed mellem observatørerne. Cohen's Kappa viste en aktuel enighed i intervallet fra 59,4-96,9%, og en styrke af enigheden fra dårlig til nærmest perfekt.

Begrænsninger: Begrænset antal af deltagende heste i projektet.

Konklusion og potentiel relevanthed: Gruppen af heste med ikke-helende mavesår, og med en stigning i RHpE scoren, fra den initielle- til kontrolundersøgelsen, var alle diagnosticeret med mavesår i kirteldelen. Selvom heste med en RHpE score ≥8 og samtidigt mavesår, viste unormal adfærd, kunne vi ikke bekræfte en signifikant sammenhæng, grundet det lave antal af heste.

Nøgleord: Hest, muskuloskeletal smerte, adfærd under ridning, adfærd under opsadling, mavesår.

2. Introduction

Equine gastric ulcer syndrome (EGUS) is a common disease and has been reported in several studies with a high prevalence [5,6]. The anatomical localization of gastric ulcers is described using the terms equine squamous gastric disease (ESGD) and equine glandular gastric disease (EGGD) [4]. ESGD and EGGD are graded using a grading system that considers anatomical location, distribution, severity, and appearance [31]. Some of the clinical signs associated with EGUS may include reduced performance [19,24,25], girthing pain and abdominal pain [24,25] changes in rideability [25], and various atypical behaviors [24,25,28]. Further, EGUS can be asymptomatically for some horses [26]. Recent studies have shown a possible correlation between abnormal behavior during tacking-up and riding, stress, and pain [40,50]. Although not evaluated yet, abnormal behavior during tacking-up may be an indication of an underlying painful condition such as EGUS [52,53] or orthopedic problems [51,53]. A protocol has been developed to evaluate abnormal behavior during tacking-up [50]. Unfortunately, many owners also fail to recognize signs of abnormal behavior during tacking-up and mounting, highlighting the importance of increased awareness in the area [79].

For several decades, lameness has been identified as the most common disease in the horse population [55–58], one of the primary causes of reduced performance [55,56,58], and is also described as an economic burden for horse owners [57]. Despite the prevalence of lameness, owners, riders, and trainers often struggle to identify lameness in the ridden horse [41,42]. Therefore, the Ridden Horse pain Ethogram (RHpE) has been developed to assist owners and trainers in detecting musculoskeletal pain through 24 behavioral markers during riding [40]. A threshold at ≥8 of the 24 behavioral markers is indicative of musculoskeletal pain [40,45] and lame horses are at 10 times higher risk of displaying some of the behaviors described in the ethogram [40]. The pathophysiology of EGGD is not fully understood, however, it has previously been described to correlate with a weakened protective mechanism of the mucosa (4). Additionally, EGGD has been noted to be associated with stress under various circumstances [15,18,20]. Stress within the body may also arise because of pain, which could be hypothesized to originate from musculoskeletal pain. This study aims to explore if EGUS is triggered by musculoskeletal pain evaluated by repeated Ridden Horse pain Ethogram (RHpE) scores, behavioral scores during tacking-up, as well as both an objective lameness examination and a subjective musculoskeletal evaluation in a group of horses diagnosed with EGUS, as well as after an EGUS treatment period.

The specific hypotheses were, 1) to assess if horses with non-healing EGUS had a higher behavior score during tacking-up and no improvement in score from the initial examination, and, 2) had a higher behavior score using the RHpE score, and no improvement in score from the initial examination, and lastly, 3) to assess if a group of horses with non-healing EGUS had a higher prevalence of horses with objective lameness, and a higher prevalence of clinically relevant musculoskeletal pain at the subjective lameness examination.

3. Material and methods

3.1 Recruitment of horses

All horses participating in this thesis were scheduled for gastroscopy at the Large Animal Teaching Hospital (LATH) due to owner suspicion of EGUS in the period from July 2023 to July 2024 (Figure 1). The owners were invited to participate in this project when they were scheduled for the gastroscopy. All horses were hospitalized and examined at the LATH. Horses of any age, sex, breed, discipline, and performance level were allowed to participate in the project. All owners should have a presumption that their horses are sound and able to walk, trot, and canter while performing a standardized riding program with a duration of eight to ten minutes. Some of the participants were not able to complete the program, often due to behavioral issues. Inclusion criteria for further participation in the control examination: the horses should be diagnosed with EGUS and follow a treatment plan for a period consisting of four to six weeks. Exclusion criteria for participating in the study were horses not being rideable, for example, young horses not broken yet, horses not in continuous training, or horses who were diagnosed with lameness.

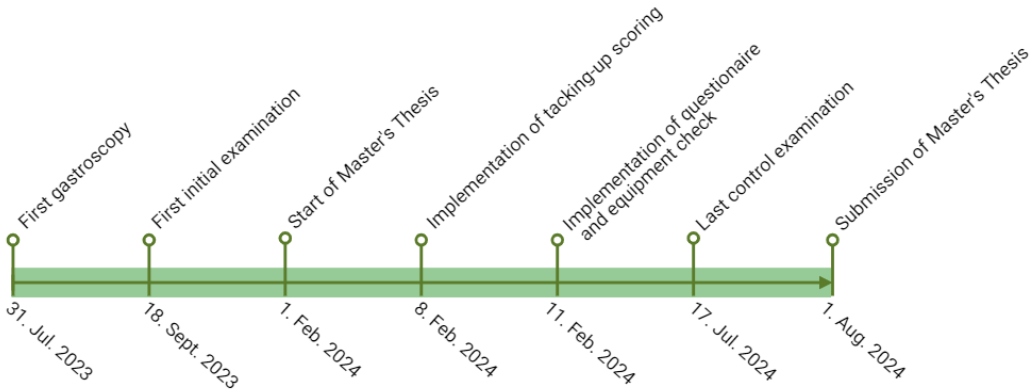

Figure 1 | Timeline illustrates the duration over which the project has been conducted, with the implementations throughout this period. It is important to note that the first gastroscopy and the first initial examinations with the standardized riding program started up before the Master's Thesis, and that tacking-up, the questionnaire, and the equipment check were implemented in February 2024. Consequently, these data were not collected for all horses included in this project.

3.2 Study protocol

The study protocol was divided into two parts and the horses were divided into 4 groups for statistical purposes (Figure 2). The initial examination includes a questionnaire, an equipment check, an objective lameness examination, a recording of the owner tacking-up the horse, and a recording of the owner riding their horse in a standardized riding program with an evaluation based on the RHpE score, further explanation is below. The horse was then starved for a gastroscopy the following day. The control examination only included horses diagnosed with gastric ulcers at the first visit and included the same procedures (objective lameness examination, recording of tacking-up and riding, and a gastroscopy) as well as a subjective lameness examination with an orthopedic veterinarian (Figure 2).

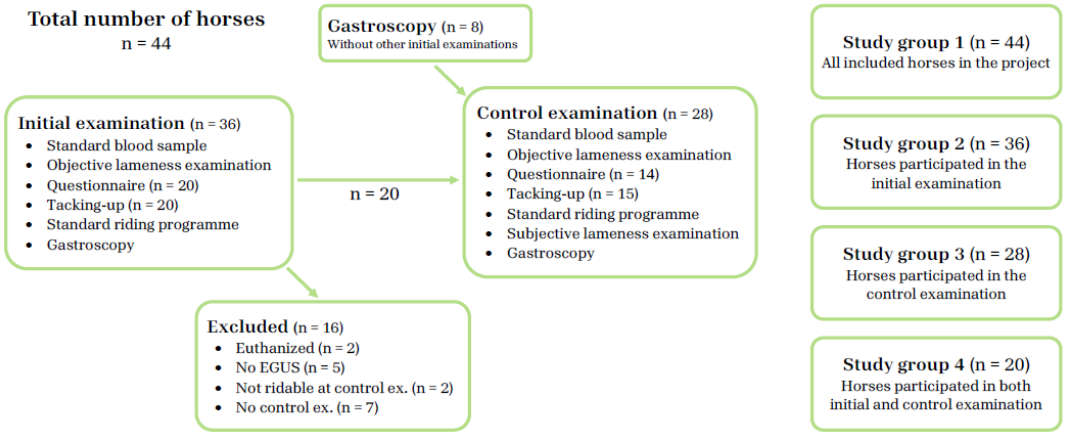

Figure 2 | A flow diagram illustrating the phases of the total number of the 44 horses included in the study. 36 horses participated in an initial examination, and 16 horses were excluded due to different circumstances. 28 horses participated in a control examination, of which 20 underwent an initial examination, and 8 horses only had a gastroscopy examination prior to the control examination. The illustration specifies how many horses participated in the different parts of the examinations and the division into four study groups. In total 64 horses underwent recording while performing the standardized riding program and 35 horses underwent recording of the tacking-up.

3.2.1 Tacking-up during video recording and questionnaire

Questionnaire and equipment check

A questionnaire and an equipment check were implemented in February 2024 to be a part of the initial examination, to collect data on the individual horse, answered by the owner. Different questions include the reason why they decided to participate in the project, the type of discipline, the amount of work, and if the owner experiences any abnormal behavior or challenges while riding and/or tacking-up, when the horse last was seen by a dentist, farrier, and saddle fitter (Appendix 1). The equipment check included an assessment of tacking-up equipment, including the bit, bridle, and saddle (Appendix 2). At the control examination, the owner was asked for any differences in behavior and riding equipment since the initial examination.

Tacking-up

In February 2024 also a tacking-up protocol was implemented at both examinations, that included that the horses were recorded while tacked-up by their owner and with their specific riding equipment. The horse was either tacked-up at the LATH before the standardized riding program or at home in their normal environment. After tacking-up, observations about the riding equipment were noted systematically based on a specific protocol (Appendix 2). The tacking-up protocol for this project was developed in cooperation and with supervision from Dr. Sue Dyson inspired by her original "Protocol for recording behavior during the approach of the horse with the tack, bridling, placement of the saddle, girthing and mounting" [50]. The protocol was simplified to contain 12 abnormal behavioral markers in total, divided into 2 scores at bridling and 10 scores at saddling (placing the saddle and girthing) (Appendix 3). The cut-off score for tacking up was defined at a threshold of ≥3, which represents the maximum number of abnormal behavioral markers that a horse was permitted to exhibit during the tacking-up process in this study.

3.2.2 Ridden Horse pain Ethogram

The Standardized Ridden Program

At both examinations, the owner had to perform a standardized riding program, developed by Dr. Sue Dyson [47]**,** by help from a person explaining the program. The program consisted of different exercises in walk, trot, and canter, and was performed in a 20x40 m indoor arena with a soft surface (Appendix 4). The owner was allowed to warm up as they usually do at home without any restrictions on time. Most of the owners rode a rising trot, but a few rode a sitting trot. All owners were allowed to use a whip and spurs if they preferred. All equipages were recorded (Panasonic 4K HC-VXF1, Panasonic Corporation, Hamburg, Germany). During the first half of the standardized riding program, the camera was placed in the corner between C and M, and between C and H during the last half of the standardized riding program (Figure 3). The camera was placed in these positions to allow seeing the horses on both reins, in different angles during straight lines, and the different sizes of circles in multiple gaits.

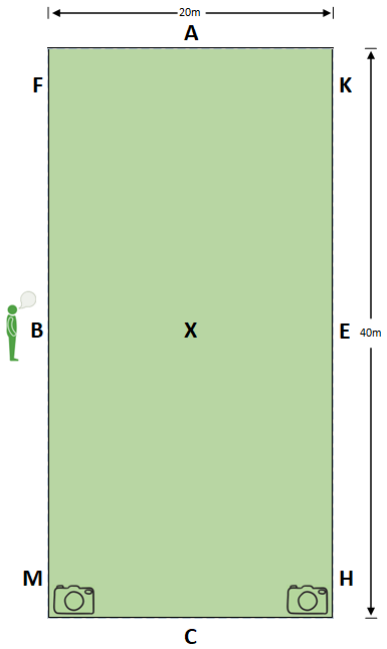

Figure 3 | The setup for the standardized riding program in the indoor arena includes positions of the camera and the speaker.

Education in the Ridden Horse pain Ethogram

The two final-year veterinary students underwent an online training course "How to Recognize the 24 Behaviors indication Pain in the Ridden Horse" [80] as an introduction before scoring the recordings. The online course lectures were given by Dr. Sue Dyson who explained in 11 lectures how to recognize different facial expressions, how the normal behavior of a sound ridden horse should be, and how to recognize the 24 behavioral markers that may be indicating that the horse is in pain. The course consisted of different video materials, exercises, and quizzes to perform.

Analyzing recording

All recordings were blinded and analyzed randomly. The standardized riding programs were analyzed in relation to the "24 Behaviors of the Ridden horse in Pain" [40] by Dr. Sue Dyson and two-final year veterinary students. The tacking-up recordings was analyzed by two-final year veterinary students.

3.2.3 Lameness assessment

Objective and subjective lameness examination

At both examinations, all horses were recorded and equipped with a lameness locator (LL) (Equinosis Q with Lameness Locator®, Equinosis, Columbia, Missouri, USA), where three sensors were placed respectively with one on the head, one on the dorsum of the pelvis, and one on the dorsal surface of the right forelimb pastern. The horses were led in hand on a straight line (40 meters) on hard surface, first at walk, then trotting up and down twice. LL can assess lameness across several degrees, categorized as mild, moderate, and severe. Additionally, it can evaluate evidence, which is categorized as weak, moderate, and strong. Horses evaluated for lameness using the LL were considered lame if one or more limbs exhibited moderate lameness or higher with moderate or higher evidence.

At the control examination, a standard lameness examination was performed by a senior orthopedic clinician. The examination consisted of collecting a history from the owner. In some cases, recordings of the standardized riding program from the day before the examination were inspected. A static examination of the horse was performed including inspection of conformation, registration of potentially relevant swellings or effusions, palpation of the horse's extremities, neck, and back including a passive range of motion test. Type of shoeing was noted, and hooves were examined including a hoof tester. Horses, equipped with the LL, were then trotted up in a straight line (40 meters) on hard surface. Furthermore, lunging on soft and hard surface in both reins was performed. Afterwards, the

horse was seen trotting in a straight line (40 meters) on hard surface again, and then all limbs underwent a full limb flexion test of a one-minute duration. Measurements from the LL were assessed. Subjective and objective findings were compared to conclude potential clinically relevant musculoskeletal findings. After undergoing a thorough examination, the horse was graded according to the American Association of Equine Practitioners (AAEP) score. Horses with a lameness score ≥2/5, were classified as clinically relevant lameness for the project evaluation. Horses that were sound but exhibited moderate back pain were also considered clinically relevant. It is important to note that in this project, lameness was only assessed for its presence (yes/no), without further diagnostic evaluation.

3.2.4 Gastroscopy

All horses that participated in this project underwent a gastroscopy after 16 hours of starvation. The gastroscopic examination was performed with a 3-meter endoscope (Endoscope, length 3 m and diameter 0.8 cm, Kruuse, Langeskov, Denmark) by an intern medicine veterinarian. The horses were sedated intravenously with detomidine (Domosedan vet 10 µg/kg, Orion Corporation, Espoo, Finland) and butorphanol (Dolorex 10 µg/kg, Intervet International B.V, Boxmeer, Netherlands). The ESGD and EGGD lesions were graded according to a grading system ranging from 0-4 [31]**,** with a cut-off of ≥2 established for diagnosing EGUS and inclusion in the project. The dose, duration, and product of treatment were decided by the individual veterinarian performing the gastroscopy, most often 4 mg/kg omeprazole once daily, administered 1 hour before feeding in the morning, was prescribed for a 4–6-week duration depending on the presence of ESGD or EGGD and severity of the lesions.

3.2.5 Blood samples

All horses participating in this project had blood samples taken at the initial and the control examination for biochemical and hematological analysis, including creatinine kinase. Additional blood samples were taken at the control examination, specifically for creatinine kinase levels 4-6 hours after completing the standardized riding program, and again the following day (approximately 12 hours later) before the subjective lameness examination. The detailed results of the blood samples analyses are not presented further in this thesis, as they are beyond the scope of this project.

3.2.6 Statistical analysis

All statistical analyses were performed using Excel (Microsoft 365, version 2405) and Sigmaplot (version 15.0). Since only few horses were included in the project, descriptive statistics were employed for most of the data (behavior, bridle, bit, saddle type, saddle challenges, saddle fitting, and number of rides per week). The horses were divided into 4 study groups for statistical purposes (Study group 1 included all horses, Study group 2 included horses participating in the initial examination, Study group 3 included horses participating in the control examination, Study group 4 included horses participating in both the initial and control examination). Data are presented as mean and standard deviations as data were normally distributed (determined by QQ-plots). For the statistical analyses, horses were divided into two groups based on the EGUS treatment response. Horses with a successful response to EGUS treatment and horses with non-healing EGUS. Data collected during tacking-up were analyzed using a Welch t-test due to unequal sample size. The data from the objective lameness examination (yes/no) and the subjective lameness examination (yes/no) were analyzed with a Fisher's exact test. For the pain expression score when ridden (RHpE), either a simple t-test or a paired t-test was used to calculate the difference between either non-healing EGUS group versus the group with a successful response to EGUS treatment, and between the initial and control examination for the same horse. For all analyses, statistical significance was defined as $p<0.05$.

The frequency of the 12 behavioral markers of the tacking-up protocol is presented in a bar chart. The RHpE scores observed at the initial and control examinations scored by Dr. Sue Dyson and the two final-year veterinary students were visually evaluated using Bland-Altman plots. The frequency of the individual 24 behavioral markers is presented in a bar chart. The inter-rater repeatability between Dr. Sue Dyson and the two final-year veterinary students was assessed using Cohen's Kappa. The agreement was graded, with the value of kappa as categorized in Table 1.

Table 1 | Cohens Kappa, agreement measures for categorical data [81].

Cohens Kappa κ	Strength of agreement
< 0.00	Poor
0.00 – 0.20	Slight
0.21 – 0.40	Fair
0.41 – 0.60	Moderate
0.61 – 0.80	Substantial
0.81 – 1.00	Almost Perfect

4. Results

4.1 Study group 1 (all included horses)

Data from 44 horses were included in the study and were collected at the LATH in Denmark from July 2023 to July 2024. The mean age was 10.8 years, and 18 (40.9%) were mares, 25 (56.8%) were geldings, and 1 (2.3%) was a stallion. Breeds included consisted of 18 (40.9%) Warmbloods, 5

(11.4%) Oldenburgs, 7 (15.9%) Icelandic horses, 9 (20.5%) Ponies, 1 (2.3%) Standardbred, 1 (2.3%) Thoroughbred and 3 (6.8%) other unspecific breeds. 27 (61.4%) of the horses were used for general pleasure riding, 11 (25%) for competition, and 6 (13.6%) for riding school.

4.1.1 Behavior changes

All owners were asked if their horse showed any behavior changes at the initial examination. Only a few of the included horses were reported not to show any changes in behavior. All other horses showed different behavior changes e.g. in riding, during tacking-up (including putting the saddle on, girth tightening, and putting the bridle on), mounting, handling, or against other horses (Table 2).

Table 2 | The distribution of behavior changes, observed by the owner, was collected from 44 horses in the period leading up to the gastroscopic examination at the Large Animal Teaching Hospital (LATH).

Variable (n = 44)	**Number** (n)	**Percentage** (%)
Behavior changes		
Riding	31	70.5
Tacking-up	26	59.1
Mounting	6	13.6
Handling	6	13.6
Against other horses	3	6.8
None	6	13.6

Out of a total of 44 horses, 28 participated in a control examination where 23 owners had reported abnormal behavior at the initial examination. These 23 owners were asked whether their horse's abnormal behavior had improved following EGUS treatment with omeprazole. Just over half of the owners reported that their horse's abnormal behavior had improved (13 horses, 56.5%) out of these 61.5% had a successful response to EGUS treatment.

4.2. Study group 2 (initial examination)

A total of 36 horses were included in the initial examination, the horses in this group had a mean age of 10.9 years, 17 (47.2%) were mares, 18 (50.0%) were geldings, and 1 (2.8%) was stallion. Different breeds in the sample consisted of 14 (38.9%) Warmbloods, 5 (13.9%) Oldenburgs, 6 (16.7%) Icelandic horses, 7 (19.4%) Ponies, 1 (2.8%) Standardbred, 1 (2.8%) Thoroughbred and 2 (5.6%) other unspecific breeds. 20 (55.6%) of the horses were used for general pleasure riding, 10 (27.8%) for competition, and 6 (16.7%) for riding school. The results for the objective lameness examination, the tacking-up, the RHpE, and the gastroscopy for the included horses at the initial examination are shown in Table 3.

Table 3 │ Summarizing the results for horses included at the initial examination including the objective lameness examination, the tacking-up score, the score evaluated from the Ridden Horse pain Ethogram (RHpE), the total number of horses with equine gastric ulcer syndrome (EGUS), and the distribution of the gastric ulcers based on their anatomical localization, equine squamous gastric disease (ESGD) and equine glandular gastric disease (EGGD). The definition of EGUS is graded as ≥2 for ESGD and/or EGGD.

Initial examination (n = 36)	**Objective lameness** (yes)	**Tacking-up score** (≥3, n = 20)	**RHpE score** (≥8)	**EGUS** (≥2)	**ESGD** (≥2)	**EGGD** (≥2)
Objective lameness (yes)	6/36					
Tacking-up score (≥3, n = 20)	1/6	9/20				
RHpE score (≥8)	6/6	3/9	21/36			
EGUS (≥2)	4/6	8/9	16/21	31/36		
ESGD (≥2)	3/6	7/9	15/21	28/31	28/36	
EGGD (≥2)	2/6	7/9	9/21	21/31	18/28	21/36

4.3. Study group 3 (control examination)

A total of 28 horses were included in the control examination, the horses in this group had a mean age of 11.0 years, 12 (42.9%) were mares, and 16 (57.1%) were geldings. Different breeds in the sample consisted of 11 (39.3%) Warmbloods, 4 (14.3%) Oldenburgs, 5 (17.9%) Icelandic horses, 5 (17.9%) Ponies, 1 (3.6%) Standardbred, 1 (3.6%) Thoroughbred and 1 (3.6%) unspecific breed. 17 (60.7%) of the horses were used for general pleasure riding, 9 (32.1%) for competition, and 2 (7.1%) for riding school. The results for the objective lameness examination, the tacking-up, the RHpE, and the gastroscopy for horses included in the control examination is shown in Table 4.

Table 4 │ Summarizing the results for horses included in the control examination including the objective and subjective lameness examination, the tacking-up score, the score evaluated from the Ridden Horse pain Ethogram (RHpE), and the total number of horses with equine gastric ulcer syndrome (EGUS), and the distribution of the gastric ulcers based on their anatomical localization, equine squamous gastric disease (ESGD) and equine glandular gastric disease (EGGD). The definition of EGUS is graded as ≥2 for ESGD and/or EGGD.

Control examination (n = 28)	**Objective lameness** (yes)	**Tacking-up score** (≥3), (n = 15)	**RHpE score** (≥8)	**Subjective lameness** (yes)	**EGUS** (≥2)	**ESGD** (≥2)	**EGGD** (≥2)
Objective lameness (yes)	6/28						
Tacking-up score (≥3), (n = 15)	1/6	8/15					
RHpE score (≥8)	6/6	6/8	15/28				
Subjective lameness (yes)	5/6	5/8	12/15	16/28			
EGUS (≥2)	1/6	4/8	11/15	4/16	15/28		
ESGD (≥2)	0/6	2/8	4/15	0/16	6/15	6/28	
EGGD (≥2)	1/6	3/8	10/15	4/16	11/15	3/6	11/28

4.4. Study group 4 (20 horses included in both the initial and control examination)

A total of 20 horses were included in both the initial and the control examinations. To be included in this group, all horses should be diagnosed with EGUS at the initial examination, participate in the

standardized riding program and the objective lameness examination at both examinations and undergo a subjective lameness examination at the control examination.

The horses in this group had a mean age of 11.0 years, 11 (55.0%) were mares, and 9 (45.0%) were geldings. Different breeds in the sample consisted of 7 (35.0%) Warmbloods, 4 (20.0%) Oldenburgs, 4 (20.0%) Icelandic horses, 3 (15.0%) ponies, 1 (5.0%) Standardbred, and 1 (5.0%) Thoroughbred. 11 (55.0%) of the horses were used for general pleasure riding, 7 (35.0%) for competition, and 2 (10.0%) for riding school (Table 5).

Table 5 │ Summarizing the proportion of lameness, tacking-up score, and the Ridden Horse pain Ethogram (RHpE) score in each group with either a successful response to equine gastric ulcer syndrome (EGUS) treatment or a non-healing EGUS at the initial and control examination.

Included in both examinations (n = 20)	**Objective lameness** (yes/total)		**Tacking-up score** (≥3/total)			**RHpE score** (≥8/total)			**Subjective lameness** (yes/total)
	Initial	**Control**	**Initial** (n = 9)	**Control** (n = 10)	**Difference between examinations**	**Initial**	**Control**	**Difference between examinations**	**Control**
Successful EGUS treatment (n = 11)	2/11	3/11	3/5	3/6	0	8/11	6/11	-2	8/11
Non-healing EGUS (n = 9)	0/9	1/9	3/4	2/4	-1	5/9	8/9	+3	3/9

4.4.1. Tacking-up scores

At the control examination, no significant difference in the tacking-up scores was found between horses with non-healing EGUS (n = 6, mean 3.2, std 2.5) and the group of horses with a successful response to EGUS treatment (n = 9, mean 2.7 std 1.8, p = 0.65).

Horses which had data from both the initial and control examination were used for these analyses. The group of horses with a successful response to EGUS treatment (n = 5), the mean tacking-up score at the initial examination was 3 (std 2.4), and at the control examination it was 3 (std 1.8), and no significant difference between the initial and the control tacking-up scores was found for this group of horses (p = 1.0). For the group of horses with non-healing EGUS (n = 4), the mean tacking-up score at the initial examination was 3.3 (std 2.6) and at the control examination it was 2 (std 1.8), and no significant difference between the initial and the control tacking-up scores was found (p = 0.45). In all observations very few horses were included as tacking-up data was only included from the study from February 2024.

4.4.2. RHpE scores

No significant difference in the RHpE score between the horses with non-healing EGUS (n = 15, mean 7.9, std 3.0) and the group of horses with a successful response to EGUS treatment (n = 13, mean 8.6 std 1.6) at the control examination was found (p = 0.42). For the group of horses with a successful response to EGUS treatment (n = 11), the mean RHpE score at the initial examination was 7.9 (std 3.2) and at the control examination 7.5 (std 2.9), and no significant difference between the initial and the control RHpE scores was found (p = 0.42). For the group of horses with non-healing EGUS (n = 9), the mean RHpE score at the initial examination was 7.1 (std 3.4) and at the control examination 8.6 (std 1.7), and a significant increase from the initial scores to the control scores was found (p = 0.04).

4.4.3. Objective lameness

No significant difference in objective lameness (yes/no) between the horses with non-healing EGUS and the group of horses with a successful response to EGUS treatment at the control examination was found (p = 0.59).

No significant difference in objective lameness (yes/no) between the initial and the control examination for all horses participating in both examinations was found (p = 0.37).

4.4.4. Clinically relevant musculoskeletal pain evaluated at the subjective lameness examination

No significant difference in subjective lameness (yes/no) between the horses with non-healing EGUS and the group of horses with a successful response to EGUS treatment at the control examination was found (p = 0.59).

4.5 Owner questionnaire

From February 2024 all the owners of the included horses were questioned about the riding equipment (n = 25). About half of the owners rode in a dressage saddle, the rest were distributed between show jumping saddles, Icelandic saddles, and treeless saddles (Table 6).

The owners were further asked if they experienced any challenges with the saddle, and if yes, they described it as saddle slip to one side, saddle slide forward, girth spams, and saddle soreness (Table 6). The majority reported that their saddle was fitted by an educated saddle fitter, while only a small number reported that their saddle was not properly fitted. Inquiring about the frequency of saddle riding per week, their answers were spread out in intervals 0-2, 3-4, or ≥5 per week, where most

owners did ride their horses in the saddle 3-4 times per week (Table 6). Besides saddle equipment, the bridle, bit, and noseband were also registered and evaluated (Table 7). Nearly all nosebands fitted the horses correctly and had a normal tightness, a few were loose, only one was too tight and one horse did not wear a noseband. The wideness of the bit fitted the horse's mouth in all cases and none of the horses had any lesions in the oral commissure.

Table 6 | Description of data related to the saddle, containing type, fitting, the owner's experiences with the saddle, and how often the saddle is used for riding per week.

Variable (n = 25)	**Number** (n)	**Percentage** (%)
Saddle type		
Dressage	13	52.0
Show jumping	7	28.0
Icelandic	3	12.0
Treeless saddle	2	8.0
Did the owner experience any challenges with the saddle?		
Yes	9	36.0
No	16	64.0
What kind of challenges (n = 9)		
Saddle slip to one side	5	50.0
Saddle slide forward	1	10.0
Girth spams	2	20.0
Saddle sore	2	20.0
Is the saddle fit by a saddle fitter?		
Yes	22	88.0
No	3	12.0
When was the saddle last fit by a saddle fitter? (n = 22)		
<6 months	13	59.1
½-1 year	3	13.6
>1 year	6	27.6
How often does the owner ride with the saddle?		
0-2 times per week	1	4.0
3-4 times per week	15	60.0
5+ times per week	9	36.0

Table 7 | Description of data related to the bridle type, tightness of the noseband, the bit type and width, and if oral lesions were observed. Definition of the noseband tightness: Loose = more than two fingers under the noseband. Normal = two fingers next to each other under the noseband. Tight = not able to put two fingers under the noseband.

Variable (n = 25)	**Number** (n)	**Percentage** (%)
Bridle type		
Flash noseband	12	48.0
Micklem	9	36.0
Drop noseband	1	4.0
Crank noseband	2	8.0
No noseband	1	4.0
Tightness of the noseband		
Loose	2	8.0
Normal	21	84.0
Tight	1	4.0
No nose band	1	4.0
Bit type		
Ordinary Snaffle	11	44.0
Eggbutt/Dee-Ring Snaffle	10	40.0
Pelham	2	8.0
Other types	2	8.0
Wide of the bit fit the horse		
Yes	25	100.0
No	0	0.0
Observation of lesions in the oral commissure		
Yes	0	0.0
No	25	100.0

4.6 Observer agreement

An overview of the 24 behavioral markers of the RHpE for all horses (n = 64) is represented in Figure 4. As well, the 12 behavioral markers of the tacking-up protocol for all horses (n = 15) are represented in Figure 5.

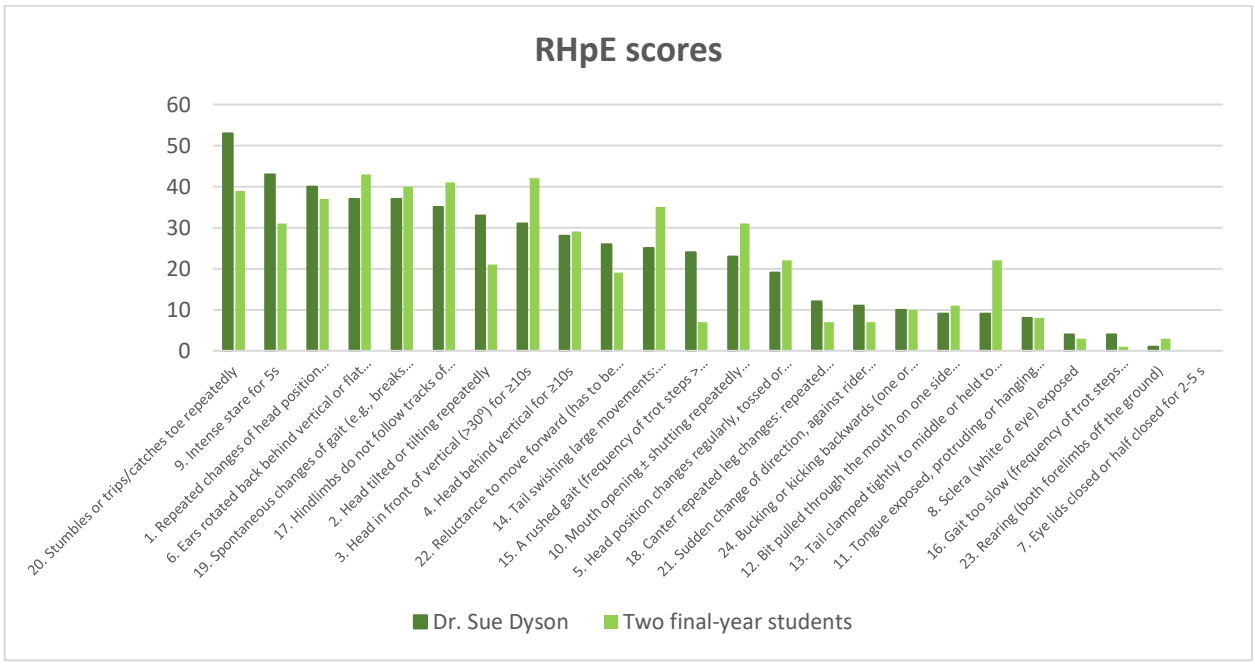

Figure 4 | Bar chart displaying the 24 behavioral markers, exhibited during riding, ranked according to their frequency with Dr. Sue Dyson's scores (dark green) and the two final-year veterinary students' scores (light green).

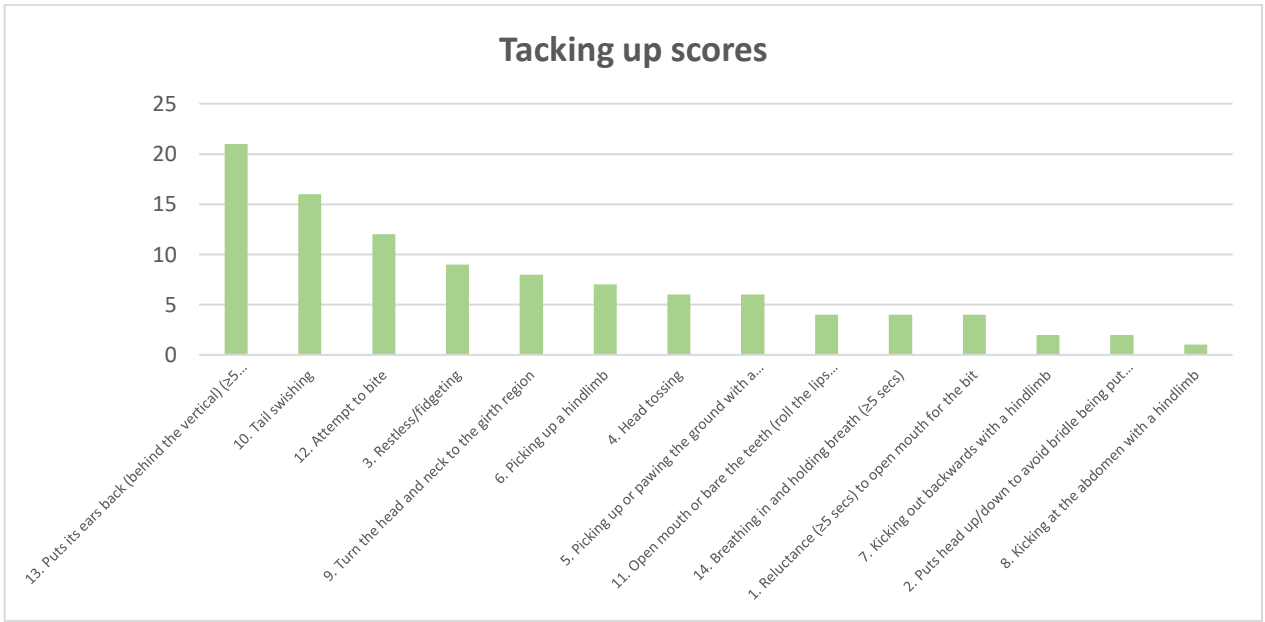

Figure 5 | Bar chart displaying the 12 behavioral markers exhibited during tacking-up, ranked after the most frequent behaviors scored by the two final-year veterinary students.

When comparing RHpE scores between Dr. Sue Dyson and the two final-year veterinary students, the Bland-Altman plot showed good agreement for the total scores for all the recordings of the standardized riding program (n = 64). All but two RHpE scores were within the 95% limits of agreement of -3.47 to 4.04, with a mean of 0.28 (Figure 6).

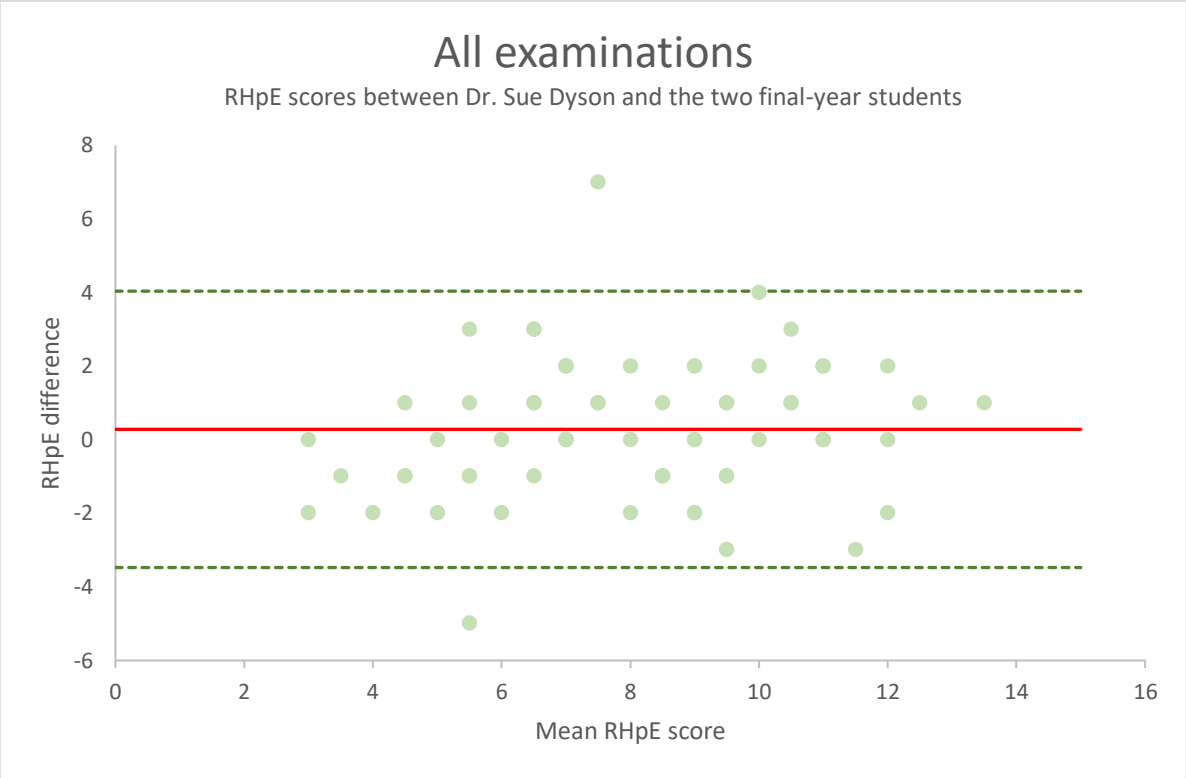

Figure 6 | Bland-Altman plot displaying the difference in RHpE scores between Dr. Sue Dyson and the two final-year veterinary students for the RHpE scores for all the recordings of the standardized riding programs (n = 64) at the initial and control examination together. The mean difference (red line) and 95% limits of agreement (dashed green lines).

When comparing the individual 24 behavioral markers between Dr. Sue Dyson and the two final-year veterinary students, for all 44 horses, there was an actual agreement ranging from 59.4-96.9% with a strength of agreement ranging from poor to almost perfect (Table 8).

Table 8 | Agreement was reached on the individual 24 behavioral markers using the Ridden Horse pain Ethogram (RHpE) scoring for 44 horses who underwent a standardized riding program during both the initial and control examinations, where they were recorded. A total of 64 videos were analyzed in a blinded and random order. The scoring was conducted by Dr. Sue Dyson and two final-year veterinary students, with the kappa value assessing their agreement level. The results are presented in order from the lowest to the highest kappa values. The behavior "eyelids closed or half closed for 2-5s" was not observed, which explains the missing kappa value.

	Behavior (According to the RHpE behavioral markers)	**Actual agreement**	**Value of κ**	**Strength of agreement**
8	Sclera (white of eye) exposed	89.1%	-0.057	Poor
16	Gait too slow (frequency of trot steps <35/15s); passage-like trot	92.2%	-0.026	Poor
13	Tail clamped tightly to middle or held to one side	67.2%	0.154	Poor
2	Head tilted or tilting repeatedly	59.4%	0.196	Poor
20	Stumbles or trips/catches toe repeatedly	66.2%	0.201	Poor
12	Bit pulled through the mouth on one side (left or right)	81.3%	0.290	Fair
15	A rushed gait (frequency of trot steps >40/15s); irregular rhythm in trot or canter; repeated changes of speed in trot or canter	73.4%	0.340	Fair
9	Intense stare for 5s	68.8%	0.382	Fair
5	Head position changes regularly, tossed or twisted from side to side, corrected constantly	76.6%	0.447	Moderate
17	Hindlimbs do not follow tracks of forelimbs but deviated to left or right; on 3 tracks in trot or canter	75.0%	0.486	Moderate
23	Rearing (both forelimbs off the ground)	96.9%	0.488	Moderate
6	Ears rotated back behind vertical or flat (both or one only) ≥5s; repeatedly lay flat	78.1%	0.538	Moderate
11	Tongue exposed, protruding or hanging out and/or moving in and out	90.6%	0.571	Moderate
1	Repeated changes of head position (up/down)	79.7%	0.577	Moderate
19	Spontaneous changes of gait (e.g., breaks from canter to trot to canter)	79.7%	0.577	Moderate
21	Sudden change of direction, against rider direction; spooking	90.6%	0.615	Substantial
22	Reluctance to move forward (has to be kicked ± verbal encouragement), stops spontaneously	82.8%	0.628	Substantial
3	Head in front of vertical (>30º) for ≥10s	82.8%	0.660	Substantial
14	Tail swishing large movements: repeatedly up and down/side to side/circular; during transitions	84.4%	0.694	Substantial
18	Canter repeated leg changes: repeated strike off wrong leg; change of leg in front and/or behind; disunited	92.2%	0.695	Substantial
10	Mouth opening ± shutting repeatedly with separation of teeth, for ≥10s	85.9%	0.701	Substantial
4	Head behind vertical for ≥10s	89.1%	0.779	Substantial
24	Bucking or kicking backwards (one or both hindlimbs)	96.9%	0.872	Almost perfect
7	Eyelids closed or half closed for 2-5s	100.0%	-	-

5. Discussion

This study included horses with an owner suspicion of EGUS, and some with riding issues, but where all the owners thought the horse to be musculoskeletal sound. This EGUS suspicion was based on several different, often behavioral issues; mainly occurring during riding and tacking-up. Previous studies have identified behavioral issues associated with EGUS, including girthing pain, abdominal pain, and poor performance [24,25]. This is in accordance with several of the issues reported by the owners participating in this study. The RHpE score included to evaluate horses in this study, was originally developed to assess musculoskeletal pain based on 24 behavioral markers [40]. Several of these observed behavioral issues are co-existing for both musculoskeletal pain and EGUS. An overlap between abnormal behavior due to EGUS and musculoskeletal pain is clear, but whether these two extremely prevalent disease syndromes can be distinguished based on behavior in different situations, has not yet been investigated.

Behavior during tacking-up has been limitedly researched, although behavioral issues during tacking-up have been reported to be highly prevalent in EGUS horses [53]. Only one study group has published on tacking-up, the first study included an extensive tacking-up protocol [50], that has been used as inspiration for the protocol developed and applied on horses included in this study. In our study, a cut-off of ≥3 abnormal behavioral markers was deemed to be associated with too much abnormal behavior. This cut-off needs further validation but was set with inspiration from the previous study [50]. The same study concluded that abnormal behavior during tacking-up including chomping at the bit, ears back, intense stare, biding, and tail swishing are common problems and may be related to stress and pain [50]. Likewise, the same group published a study looking at owner recognition of abnormal behavior. This study revealed that a large population of horse owners interpreted abnormal behavior during tacking-up and mounting as normal behavior for their horses [79]. In our study, a shorter and more user-friendly tacking-up protocol was developed and applied to included horses. Unfortunately, only a few horses were included, and therefore no trend could be found in the results. The score was easy to apply both in real life and on the recordings. 6/9 horses at the initial examination and 5/10 at the control examination showed ≥3 abnormal behaviors during tacking-up. Future studies should be conducted to focus on this important welfare point.

In conjunction with obtaining the tacking-up data, a large part of the horse owners reported that they had focused on saddle fitting, despite this, the owners still experienced challenges with their saddle while riding. Our observations align with several studies that suggest saddles should be refitted

multiple times per year [82,83]. Our findings indicate that every six months may still not be sufficient, as changes in workload [84], and season variations [82] can impact the dimensions of the horse's back, potentially necessitating more frequent adjustments to the saddle fit. A poorly fitted saddle has been shown to cause abnormal behavior associated with back pain [50,76].

The assessment of the bit and bridle revealed that all horses were equipped with appropriately sized bits and no oral lesions were found in the oral commissure. Oral lesions are prevalent in ridden horses [85,86] with increased frequency correlating with higher levels of competition [86,87]. Our study was predominated by pleasure horses, with only a few competition horses at a relatively low level, possibly explaining the absence of observed lesions. Additionally, our assessment focused solely on the oral commissure. Furthermore, the prevalence of oral lesions was linked to horses fitted with tight nosebands due to pressure on the buccal side of the mouth against the teeth [88]. Since only one horse in our study wore a tight noseband, this may also account for the lack of observed lesions.

Previous studies have identified the RHpE score as an indicator of musculoskeletal pain in horses [40]. However, based on published abnormal behavior in the EGUS literature, EGUS may be able to affect the RHpE score, although not scientifically validated yet, as many of the 24 behavioral markers overlap between musculoskeletal pain and EGUS [24,25,28,40,45,74,75]. Especially RHpE behavioral markers, such as tail swishing, bucking, ears rotated back, intense stare, and reluctance to move forward, are likely also present in horses diagnosed with EGUS. As an example, EGUS affects equine behavior, leading to reduced performance [19,24,25], changes in rideability [25], and behavioral changes [24,25,28]. In our study, 15 horses in total had a RHpE score ≥8, of which 12 were diagnosed with subjective lameness, 6 with objective lameness, and 11 with EGUS. Co-existence of lameness and EGUS occurred in 4 horses.

Of the 16 horses that exhibited subjective lameness, only 12/16, had a RHpE score ≥8, but all horses in our study that were objective lame on both the initial examination (6/6) and control examination (6/6), had an RHpE score ≥8. Previous research described that a score of ≥8 is the most reliable for identifying musculoskeletal pain with a sensitivity of 0.86 [45]. This discrepancy between the objective and subjective lameness, as well as the RHpE score, might be caused by the more thorough subjective lameness examination which includes multiple elements, while the objective examination performed in a straight line cannot be regarded as sufficient in isolation [42]. As well as the determination of the threshold on the LL that for this study was defined only to include horses as lame if the LL evaluated moderate lameness or higher, with moderate or higher evidence. This criterion may

have been too stringent a cut-off value, which could explain why more objectively assessed sound horses have a RHpE score ≥8. Furthermore, as previously pointed out, the high RHpE score, may also be attributed to horses that have concurrently been diagnosed with EGUS, where the pain associated with these ulcers may contribute to the exhibition of abnormal behavior in horses during riding. Another reason for the discrepancy could be attributed to an uncertainty of the RHpE score, as the natural light from windows compromised the video quality, potentially obscuring abnormal behaviors. This limitation could have been mitigated by conducting live assessments.

Out of 11 horses diagnosed with EGUS who had an RHpE score ≥8, 7 were sound and not diagnosed with a musculoskeletal issue. It is generally suspected that EGUS may influence a horse's evaluation during riding, resulting in a RHpE score ≥8. Further studies are needed to investigate the relationship between the presence of both EGUS and/or musculoskeletal pain, as horses exhibit overlapping abnormal behaviors [24,25,28,40,45,74,75]. Based on our study, consideration should be given to whether horses with RHpE scores ≥8 are valid for evaluation of musculoskeletal pain if they also are diagnosed with EGUS. It may be necessary to exclude the presence of EGUS before using RHpE scores to evaluate musculoskeletal pain and vice versa. This should be investigated more thoroughly with a larger sample size to ensure if RHpE scores are falsely elevated due to EGUS.

Out of the 7 horses who were sound and had a RHpE scores ≥8, 4/7 were diagnosed with ESGD, and 6/7 were diagnosed with EGGD, whereas three of them were diagnosed with both ESGD and EGGD. Due to the small number of horses in our study, we are not able to comment on whether the RHpE score ≥8 is specifically affected by the presence of either ESGD or EGGD. However, we noted that 6 out of 7 sound horses with RHpE score ≥8 were concurrently diagnosed with EGGD, some of which also had ESGD. Nevertheless, it cannot be ruled out that EGUS, ESGD, or EGGD may influence the RHpE score. Before more specific conclusions can be drawn regarding this suspicion, additional studies with a larger cohort are necessary.

We demonstrated a significant increase in the RHpE score, between the initial and control examination for horses with non-healing EGUS, with an increase of 1-2 scores in their average RHpE score from the initial to the control examination. Out of the 9 horses with non-healing EGUS 7 of them had a RHpE score which increased from the initial to the control examination. All 7 horses were diagnosed with EGGD, whereas 2 of them also were diagnosed with ESGD. This observation is likely attributable to the persistent pain of the EGUS, as 8 out of the 9 horses had a RHpE score ≥8, and only 3 out of the 9 horses with non-healing EGUS were evaluated with a subjective lameness.

The Bland-Altman plot showed good agreement between Dr. Sue Dyson and the two final-year veterinary students for all but two scores. This is shown by the mean difference (bias) which is close to 0 and by the randomly scattered data points close to the mean difference. There is no trend in the data points, which indicates that the agreement between Dr. Sue Dyson and the two final-year veterinary students is throughout the scale of scores. “Stumbles or trips/catches toe repeatedly” was the most frequent behavioral marker in this study, this can be due to the surface condition in the arena throughout the project, which unfortunately ranged from wet and hard to very deep and dry. Such variability complicates the horse’s ability to avoid toe drag whenever the surface is deep [43]. However, based on previous studies the following behavioral markers stumbles or trips/catches toe repeatedly, intense stare, bit pulled through and a rushed gait are certainly more difficult to identify for an untrained assessor compared to a trained assessor [45]. This aligns with the observation that these four behavioral markers are positioned by the lowest levels of actual agreement, exhibiting only poor and fair strengths of agreement based on Cohen’s Kappa calculations. Additionally, it may elucidate why there is greater consensus on certain other scores that are arguably easier for untrained assessors to recognize. In the evaluation of Cohen’s Kappa values, it has been observed that the strength of agreement ranges from poor to fair in several instances, despite a high actual agreement. This can be attributed to the fact that the kappa value decreases as the observers agree more frequently that a behavioral marker is absent. Consequently, Cohen's Kappa has a limitation that does not account for the agreement regarding the behavioral markers that are not expressed in the horses.

5.1 Limitations

The most important limitation of the project was the insufficient number of horses included, which precluded the conclusion of significant results. This was due to the low recruitment of horses and the fact that some participants only took part in one of the two examinations. Additionally, the conditions in the indoor arena were variable. The lighting in the arena varied due to changing seasons, which affected the quality of the individual recordings of the horses. Moreover, the surface in the arena was inconsistent due to a lack of structured maintenance. Concerning the performance of the standardized riding program, some owners did not perform the program in rising trot, and some owners or horses were unable to complete the entire program, which limited the accurate scoring of the 24 behavioral markers in the RHpE. The delayed implementation of tacking-up recordings, a questionnaire, and an equipment check was a limitation for the results, as it contributed to reducing the sample size for those groups.

5.2 Conclusion

In conclusion, a significant increase in the RHpE score with 1-2 scores was observed between the initial and the control examinations for horses with non-healing EGUS. All horses in this group were continuously diagnosed with EGGD at the control examination, whereas ESGD showed a better response to EGUS treatment with 5/7 horses with a healing ESGD at the control examination. However, further investigations with a larger sample size are required to elucidate the potential relationship between abnormal behaviors in horses with a RHpE score ≥8 and co-existing EGUS, or even EGGD, before drawing definitive conclusions. Likewise, further investigations on abnormal behavior during tacking-up and musculoskeletal pain in association with EGUS, are needed, as no significant results could be demonstrated due to the low sample size. There is overall strong agreement between Dr. Sue Dyson and the two final-year students regarding the use of the RHpE scoring system. It is assessed that with appropriate training, the implementation of this system can be improved, facilitating its application in daily veterinary practice with a focus on abnormal behavior in ridden horses.

To enhance the welfare of ridden horses in the future, it is crucial to draw attention to horses exhibiting abnormal behaviors during tacking-up and riding, as these behaviors may overlap with manifestations of both EGUS and musculoskeletal pain. An increasing focus on abnormal behaviors may, in the future, help mitigate the risk that riders, owners, trainers, and veterinarians overlook the possibility of co-existing conditions.

6. Perspective

For the project, a larger participation of horses would have been desirable to achieve a larger sample size for obtaining significant results. A desired sample size of 50 horses participating in both the initial and the control examination would be optimal for achieving statistically significant results. This sample size is determined based on a power of 0.8, a significance level of 0.05, a clinical relevance difference of 2 scores, and a standard division of 2.5. With a larger sample size, it may be possible to categorize the horses into four distinct groups. Group 1 includes horses diagnosed with EGUS, Group 2 includes of horses experiencing musculoskeletal pain, Group 3 includes horses exhibiting both EGUS and musculoskeletal pain, while Group 4 serves as a control group without any diagnosis of either EGUS or musculoskeletal pain. This would allow for an assessment of whether RHpE can be used to detect EGUS, validate its relation to musculoskeletal pain, and determine if RHpE can also detect lameness in the presence of co-existing EGUS.

Ideally, all horses and owners included in the study should demonstrate proficiency in all exercises outlined in the standardized riding program. Also, the horses should possess the necessary education and training to perform correctly on the bit on both reins in all three gaits, thus avoiding inaccuracies in scoring, such as when the horse is above or behind the bit. Failure to exhibit proficiency in all three gaits will result in the inability to assess all 24 behavioral markers accurately. These measures are essential for ensuring the standardization of the RHpE score evaluation process. As several owners have opted out of participating in the control examination, an offer with a discounted price for horses undergoing both initial and control examinations to encourage attendance at both examinations without financial barriers.

Based on the analysis of the standardized riding program recordings, it is evident that the lighting conditions in the indoor arena have undergone variations due to seasonal changes. The presence of sunlight streaming through the windows has had a detrimental impact on the quality of the recordings, making it challenging to accurately assess certain aspects of the horse's movement when the sunlight directly interfered with the camera's field of view. A potential solution for the future project would involve the installation of curtains to avoid the adverse effects of direct sunlight on the recording process.

Considering recording the tacking-up at the horse's home stable, rather than at the LATH, as several horse owners have noted their horse exhibited more abnormal behavior at home compared to when they are at the hospital in an unfamiliar environment. Therefore, it is advisable to develop a standardized protocol for recording the tacking-up in a familiar and normal environment. During the work of our thesis, we have had several considerations regarding whether a more comprehensive lameness examination should be included in the initial examination. The advantage of this is that a more thorough lameness examination is needed to better utilize data for comparing potential lameness with data from the control examination. This would allow for comparing changes in lameness, RHpE scores, and EGUS between the initial and control examination more truthful. On the other hand, the disadvantages include the potential exclusion of certain horses from the control examination if musculoskeletal pain is detected during the initial examination, as this would presumably result in the horse being unable to participate in the standardized riding program at the control examination.

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9. Appendix

1. Questionnaire
2. Equipment check
3. Tacking-up protocol
4. Standardized riding program

Appendix 1

Questionnaire

Horse name:

History

How long time have you had the horse?	
How is the horse' environment?	Housing: Paddock: Feeding:
Reason for gastroscopy and participation in the project	
Use of the horse?	
Are there any problems/challenges during riding?	
How is the horse trained in a normal week?	
Rider's level	1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ Beginner Professional

Teeth

When was the last time for a dental check?	

Hoofcare

Shooing/barefeet	
When was the last time for shooing/trimming?	
Has there been any changings in the shooing/trimming?	

Eyes

Visible sclera at rest	

Appendix 2

Equipment check

Horse Name:

Bridle and bit

Observation of lesions in the oral commissure			
Bridle type			
Tightness of the noseband	Loose ☐	Normal ☐	Tight ☐
Bit type			
Wide of the bit			
Changes in bridle/bit?			

Saddle

Is the saddle fitted by a saddle fitter?	
When was the saddle last by a saddle fitter?	
Are there any challenges with the saddle?	
How often do you ride with a saddle?	
Do you ride with any other saddles?	

Observation of the saddle

Type of saddle	
Tree too wide	
Space around the withers - Narrow gullet/pommel too low	
Tree too long (T8-T18)	
Balance - Seat tips forwards/backwards	
Dorsoventral movement of the back of the saddle	
Panel bridging	
Other observations	

Appendix 3

Tacking-up scoring			
When the bridle is put on, does the horse display any of the following behaviors?			
1	Reluctance (≥5 secs) to open mouth for the bit	Yes	No
2	Puts head up/down to avoid bridle being put on	Yes	No
When the saddle is placed on the horse's back and the girth is done up, does the horse show any of the following behaviors?			
3	Restless/fidgeting	Yes	No
4	Head tossing	Yes	No
5	Picking up or pawing the ground with a forelimb	Yes	No
6	Picking up a hindlimb	Yes	No
7	Kicking out backwards with a hindlimb	Yes	No
8	Kicking at the abdomen with a hindlimb	Yes	No
9	Turn the head and neck to the girth region	Yes	No
10	Tail swishing	Yes	No
11	Open mouth or bare the teeth (roll the lips back to expose closed teeth) (≥5 secs, or more than 3 times)	Yes	No
12	Attempt to bite	Yes	No
13	Puts its ears back (behind the vertical) (≥5 secs, or more than 3 times)	Yes	No
14	Breathing in and holding breath (≥5 secs)	Yes	No

Appendix 4

The Standardized Riding Program

Camera position: MC		
1	A A-A-C	Working trot One and a half circuits around the periphery
2	C	Circle - 20 meters, working trot
3	C (X)	Circle - 20 meters, working trot At the middle transition to walk 5 steps and transition to working trot
4	C-A	Working trot along the side
5	A A-C	Transition to working canter right rein Working cantor one and a half circuits around the periphery
6	C	Circle - 20 meters, working cantor
7	C	Transition to working trot
8	B-E-B B BX X XE E EX X XB BA	Figure of eight maneuvers, working trot Circle - 10 meters Half circle (right) Straight line at the middle, change rein from right to left Half circle (left) Circle - 10 meters Half circle (left) Straight line at the middle, change rein for left to right Half circle (right) Working trot
9	A	Transition to walk
10	A-X-C	Two-loop serpentine
Camera position: HC		
11	C C-C	Working trot One circuit around the periphery
12	C	Circle - 20 meters, working trot
13	C (X)	Circle - 20 meters, working trot At the middle transition to walk 5 steps and transition to working trot
14	C-A	Working trot along the side
15	A A-A-C	Transition to working canter left rein Working cantor one and a half circuits around the periphery
16	C	Circle - 20 meters, working canter
17	C C-E	Transition to working trot Working trot along the side
18	E-B E EX X XB B	Figure of eight maneuvers, working trot Circle - 10 meters Half circle (left) Straight line at the middle, change rein from left to right Half circle (right) Circle - 10 meters
19	B-A	Working trot along the side
20	A A-M	Transition to working canter right rein Working canter along the side
21	M-X-K X	Diagonal change of rein, working canter Transition to working trot
22	A A-H	Transition to working canter left rein Working canter along the side
23	H-X-F X	Diagonal change of rein, working canter Transition to working trot
24	A A-E	Transition to working canter right rein Working canter along the side
25	E E-C	Transition to working trot Working trot along the side
26	C	Transition to walk