Sveriges lantbruksuniversitet
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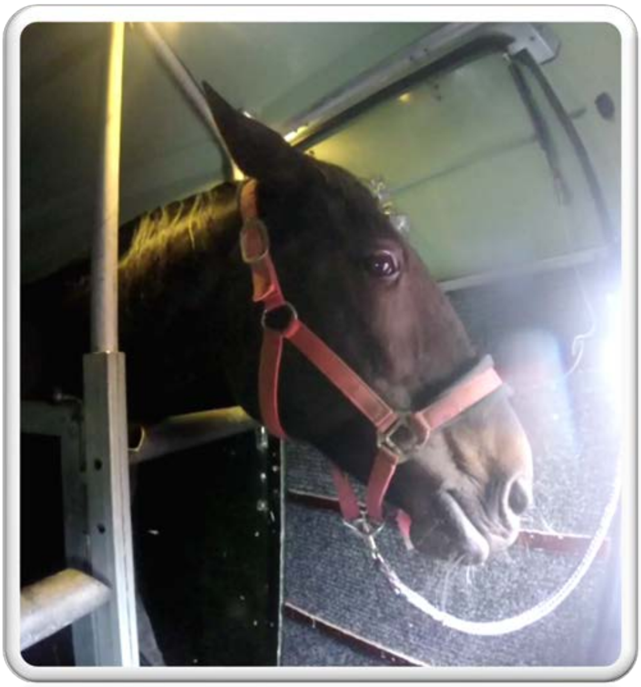
**Faculty of Veterinary Medicine
and Animal Science**
Department of Clinical Sciences

Changes in facial expressions during short term emotional stress as described by a Facial Action Coding System in horses

Johan Lundblad



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Changes in facial expressions during short term emotional stress as described by a Facial Action Coding System in horses

En beskrivning av förändringar i ansiktsuttryck när hästar utsätts för kortvarig emotionell stress

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SUMMARY

Animals subjected to stress is a well-known issue that has to be taken into account when working in a clinical environment. A stress response is closely related to several physiological and behavioural effects that could potentially mask symptoms of pain. So far, research has focused on evaluating stress using ethograms or physiological indicators. However, little has been focused on using facial expressions when evaluating stress. This is important because only a little research regarding facial expressions of pain has considered the potential of other emotions affecting the readings. Therefore, this thesis discusses the concepts of pain and stress and how they work together, as well as methods for objectively evaluate pain and stress, using literature available today.

An observational semi-randomized pilot study containing six horses was performed with the objective to generate hypotheses and methodology for further studies in the area. The horses were video filmed in their own stable without observers approximately sixty minutes to collect a baseline. These films were annotated in ELAN software using the Equine Facial Action Coding System. These horses were then subjected to an emotional stressor, transportation by road, and filmed in the transport for approximately twenty minutes. During baseline filming and filming during transportation, heart rate was collected using an equine electrode transmitter that was applied on the horses' heart silhouette. This was done to get an indication of the susceptibility to stress of the horses measured. All horses showed an increase in heart rate during transportation. The changes in the horses' facial expressions were shown through descriptive statistics with some significance testing.

The transported horses overall showed tendencies to tension in certain facial muscles, since the frequency of nostril dilation ($p=0.005$) and upper eyelid raiser ($p=0.02$) increased during transportation. The transported horses also experienced a more extrovert behaviour regarding blinking, ear- and head movements. The frequencies of these movements increased during transportation. This thesis concludes that horses do express an increased frequency of certain facial expressions during emotional stress. It is anticipated that these may potentially affect pain evaluation using the equine pain face. However, further studies with a larger population is needed to draw further conclusions.

SAMMANFATTNING

Ett välkänt problem som måste tas i beräkning när djur undersöks i en klinisk miljö är det faktum att de utsätts för emotionell stress. Stress ger upphov till en rad fysiologiska svar samt beteendeförändringar hos djuret och dessa kan potentiellt maskera symptom på smärta. I dagsläget har forskning inom området fokuserat mycket på etogram och fysiologiska indikatorer för stressutvärdering. Dock har lite fokus legat på hur stress kan utvärderas genom avläsning av ansiktsuttryck. Detta är viktigt eftersom forskningen rörande ett "smärtansikte" hos häst till låg grad har inkluderat andra emotionella stadier hos hästen när smärta utvärderas genom ansiktsuttryck. Därför fokuserar denna rapport på att diskutera koncepten smärta och stress samt hur dessa påverkar varandra. Metoder för att objektivt mäta smärta och stress diskuteras likväl.

En delvis randomiserad observationsstudie innehållande sex hästar presenteras. Målet med pilotstudien är att generera hypoteser och metodologi för vidare studier inom området. Hästarna videofilmades i deras egen box utan observatörer närvarande under sextio minuter för att inhämta basvärden. Dessa filmer annoterades i programvaran ELAN med hjälp av verktyget Equine Facial Action Coding System. Efter det exponerades hästarna för emotionell stress, transport, och filmades under transporten i ungefär tjugo minuter. Under basvärdes-filmningen och filmning under transporten samlades mätvärden för hjärtfrekvens med hjälp av en elektrod men en inbyggd sändare applicerad på huden över hastens hjärtsiluett. Detta gjordes för att visa på mottaglighet av stress hos hästarna. Alla hästarna visade på en ökning av hjärtfrekvensunder transport. Förändringarna i hästarnas ansiktsuttryck visades med deskriptiv statistik med vissa signifikanstester.

Hästarna visade generellt på tendenser till att uttrycka vissa ansiktsuttryck under transport. Frekvensen av dilatation av näsborren (p=0,005) och upplyft av övre ögonlocket (p=0,02) ökade under transport. Hästarna visade även på ett ökat extrovert beteende illustrerat av ökad frekvens av blinkningar samt öron- och huvudrörelser under transport. Denna rapport konkluderar att det finns tendenser till att hästar visar särskilda ansiktsuttryck under stress och att dess ansiktsuttryck eventuellt kan påverka avläsningen av hästens "smärtansikte". Dock krävs vidare studier med en större population för att dra slutsatser som kan appliceras på en större grupp hästar.

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INTRODUCTION

It is well known that one of the core purposes of the veterinarian profession is to prevent animals from suffering. It is one of the foundations for a good animal welfare and ethical treatment of our companion and production animals. When discussing animal welfare, one tends to bring up the five freedoms. While one's mind often trail to feeding and housing conditions when reading these, one should not forget that pain and stress takes up two of the five freedoms. "Freedom from fear and distress" and "Freedom from pain, injury and disease" are point four respectively point five of the five freedoms (Gaynor & Muir, 2014). Stress is triggered by stressors e.g. pain, discomfort, social isolation, exercise which all produce an input to the central nervous system. All of these may induce a stress response in the animal with the purpose of maintaining homeostasis. The stress response then exerts a physiological response which purpose is to maintain body functions during a threatening event. For a flight-animal such as the horse this response would come when the animal perceives a threat (Gaynor & Muir, 2014).

Emotional stress is a factor the clinician daily must take in to account when treating animals. Horses regularly experience these types of stress when treated, since few procedures is completely stress-free. Even though many horses are treated at home, there are situations where the horse require to be transported to a clinic for treatment. Transports themselves can be stressful for a horse (Schmidt *et al.*, 2010; Tateo *et al.*, 2012; Ishizaka *et al.*, 2017). If one also adds up the facts that the horse most likely will be taken from its flock, to a new environment with new smells, in addition to exposure from different procedures the total amount of stress one individual could experience should be relatively high. The strain on the clinicians' ability to properly evaluate pain is high and could possibly be clouded by several factors.

It is not hard to understand why emotional stress has such an impact on the clinicians' ability on evaluating pain. Both stress and pain is a biological response to a stressor that threatens the body's homeostasis and furthermore both shares several of the same nervous pathways (the sympathetic nervous system) and exerts similar effects on the body as a whole (Muir, 2013). Therefore, it is highly relevant to include stress when constructing methods for pain evaluation. In the worst-case scenario, stress could potentially mask (partially or completely) symptoms of pain. Furthermore, it is relevant for veterinarians and veterinary technicians to recognize stress in a clinical environment as these settings often induce stress in patients. Since stress exerts effects on the immune system (Webster Marketon & Glaser, 2008) it is in our best interest to recognize and prevent stress in patients, both from a clinical standpoint as well as from an animal welfare perspective.

We know that pain induces tension in specific facial muscles (Gleerup *et al.*, 2015). Since there are so many similarities between stress and pain on a physiological level there is a reasonable good possibility that those similarities exist when evaluating "pain face". In that case, could stress be an aggravating factor when interpreting pain face? When measured using the Horse Grimace Scale (HGS) (Dalla Costa *et al.*, 2017) it suggested not. However, the use of EquiFACS (The Equine Facial Action Coding System) could help draw more detailed conclusions regarding the Equine Pain Face. To be able to answer that question one must first know whether or not horses express emotional stress through facial expressions. Therefore, this pilot study aims to describe the facial expressions observed during short term emotional stress using a systematic and objective approach. An objective method, EquiFACS, will be used in order to discover and determine the fascial expressions of emotional stress in horses without pain. The hypothesis is that horses, above coping behaviors and physiological factors, also expresses stress that can be detected with the use of facial action units and actions descriptors. Furthermore, it was hypothesized that the equine pain face and stress face share similar FACS. This will lay as a solid ground for further studies regarding a more specific evaluation of pain in horses using behavioral methods as well as allow us to evaluate stress, both during clinical conditions and as an indicator for animal welfare.

The thesis will discuss the concepts of stress and pain and how they work together in more detail. Methods to objectively score facial expressions are also reviewed and how they can be used to evaluate pain and stress. Finally, a pilot study with six horses subjected to an emotional stressor, transportation, is presented.

LITERATURE REVIEW

What is stress?

As earlier mentioned, stress is a bodily function in response to stressors. It is both a subjective coping mechanism, which varies greatly among individuals (Ijichi *et al.*, 2013), and a system definition of a potential threat to the homeostasis. To properly define stress, one need to determine the differences between a stressor and a stress response. In order for a stress response to be induced in an individual a potential threat to that individuals homeostasis must occur (Chapman *et al.*, 2008). Such an event could be a horse observing something it could perceive as a threat. Such a threat can be a sensory stimulus, as well as a physical injury or damage to internal structure, e.g. a visual threat, a wound or even surgery during anesthesia. Such an event is called a stressor. The individuals' response to that stressor is called a stress response, e.g. increase of heart rate or release of epinephrine in the bloodstream. The purpose of this response being to maintain homeostasis (Chapman *et al.*, 2008) and make the individual cope with the changes in the environment.

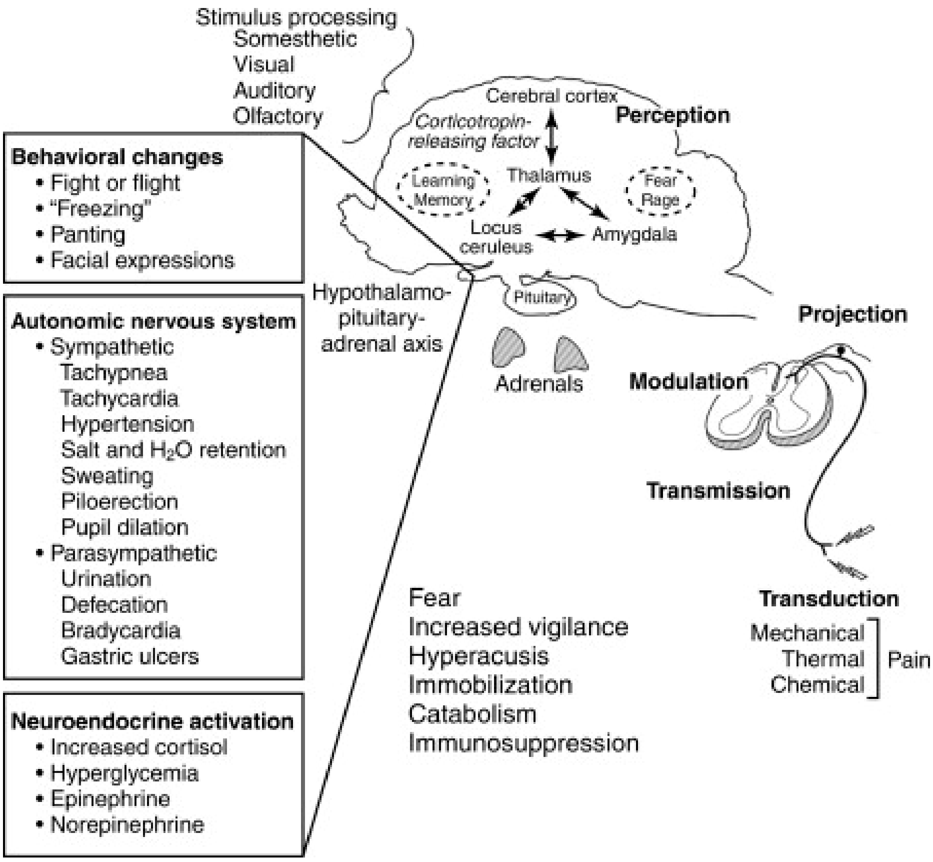

Figure 1. *Illustration of a stress response. Reprinted from (Gaynor & Muir, 2014) with permission from Elsevier*

Stress can be divided into anxiety and fear (Muir, 2013). When a body's fight and flight-mode is activated the stress response is mostly conducted through fear, therefore protecting the body from an ongoing danger. A response which in evolutionary perspective has proven to be positive (Collins, 2016). However, the anticipation of a dangerous stressor instead triggers an anxiety-driven response. In the wild, horses who carried this trait got better at anticipating dangers and prepared the body for flight (Muir, 2013). The effects of short-term stress are necessary, not just only in an evolutional standpoint, but also in a clinical standpoint. When performing surgery, the release of cortisol has proven that stress is necessary for certain body functions (Taylor, 1989). Chronic or long-term stress however, has been shown to carry out negative effects, since the body is put in a strain in order to restore a shift in homeostasis, which might not even be there anymore. Long-term exposure to these strains carry out negative effects to the body, as well as, on a bigger scale, animal welfare problems (Pawluski *et al.*, 2017).

Stress is a state in which several systems of organs are involved (Chapman *et al.*, 2008), with or without the involvement of pain. Primarily, sensatory stimuli may induce the activation of the neuroendocrine axis, also called the HPA-axis (Hypothalamic-Pituitary-Adrenocortical axis) as an outcome of the release of CRF (Corticotropin Releasing Factor). This stimulation can be activated through surgical stimuli, pain, inflammation, arousal, anxiety or fear (Gaynor & Muir, 2014) to mention a few. The stimulation from the HPA-axis carry out effects on the amount of circulating hormones, metabolism and the immune system. Cortisol is released when the adrenal cortex is activated through sympathetic efferent nerves connected to the cortex. Epinephrine and norepinephrine are also secreted. Together with various pancreas hormones, they push the metabolism into a catabolic state, promoting the release of energy substrates into the bloodstream. Stress also induces changes in the immune system. This mainly involves production of cytokines (IL-1, IL-6 and TNF-A) and an acute phase response, preparing the body for eventual tissue damage or harm (Gaynor & Muir, 2014) but also more specific changes like reduced number of lymphocytes, decrease of antibodies, reactivation of latent viruses and a decreased helper/suppressor ratio (Webster Marketon & Glaser, 2008).

Physiological markers to evaluate a stress response

Clinical evaluation

Because of the activation of the sympathetic nervous system there are several clinical parameters that can be used to evaluate stress. Heart rate is the one of the parameters studied to have shown represent emotional stress in horses during road transport (Schmidt, 2010). Another tool regarding heart rate is the use of heart rate variability (HRV) which can be used to distinguish between activation of the sympathetic and parasympathetic nervous system in horses (Zebisch *et al.*, 2014). However, it is important to note that the sympathetic nervous system can be activated through several processes (e.g. pain, exercise) and most of these parameters are not specific for a stressed individual. Furthermore, there are studies that show heart rate have a week correlation with stress. For example, Munsters, *et. al.*(2013) found that heart rate and heart rate variability corresponded low with actual cortisol release in horses during airline transportation. Systolic and diastolic blood pressure has been shown to increase during stressful situations (Hydbring, 1998), however the measurements often require invasive methods that themselves can cause stress and is therefore rarely used.

Cortisol

The HPA-axis is well described and it has been proven that emotional stress induces elevated levels of cortisol in plasma (Alexander & Irvine, 1998). Cortisol has traditionally been used to measure stress (Novak *et al.*, 2013) and has been evaluated to an accurate indicator of stress (Hoffsis & Murdick, 1970). However, cortisol varies when examined which can be problematical when used in a clinical environment. Both circadian rhythm (Kirkpatrick *et al.*, 1976; Alexander & Irvine, 1998), and variations in age (Donaldson *et al.*, 2005) has been shown to affect the release of cortisol from the HPA-axis. Plasma concentrations of cortisol also elevates when the animal is exposed to pain. Ayala *et al.*, (2012) showed that cortisol-levels in horses with several acute and chronic diseases (several of which includes horses in pain) rises. Therefore showing that the axis has a central role when evaluation stressors associated with pain. Ayala *et al.*, (2012) also showed that cortisol has a bigger role in acute pain as compared to chronic pain. Finally, Cortisol-levels also elevates during physical stress making it a generally secure hormone to determine stress regardless of cause (Cayado *et al*., 2006). Cortisol can be measured in plasma/serum, saliva, feces and hair (Novak *et al.*, 2013).

Other physiological markers

When discussing catecholamines, mainly epinephrine and norepinephrine is relevant in measurements of stress (Hydbring, 1998). Since these hormones are a key component in regulating a sympathetic response they are valuable to measure but should, as with cortisol, not be measured separately in order to evaluate stress but rather be viewed as a whole with other measurements. Endorphins, such as β-endorphin has been studied in conjunction with restraint and gastric tubing in horses (Hydbring, 1998). These levels rose during the treatments but quickly returned to control levels after 30 minutes, indicating that it could be used to determine short term stress. The same study evaluated hematocrit as a possible marker with the conclusion that stressful situations could give a rise in hematocrit. β-endorphin has also been shown to rise in horses during exercise (Cravana *et al.*, 2010).

The possible relationships between stress and pain

Similarities and differences between stress and pain

The similarities between stress and pain are many as they both are systems that work closely together. For example, they both are closely linked to the autonomic nervous system, exerting their effects on the HPA-axis and the sympato-adrenomedullar system, as well as promoting the same type of hormonal and physiological response. The purpose of both systems is also the same, to maintain homeostasis in the animal. However, it is important to distinct between an emotional stress response and a stressor. Pain itself can be a stressor, causing an individual to develop a stress response. As we will discuss in this thesis, pain and stress apart from having similarities, also closely work together and the systems affects each other more than one could primarily think. As previously mentioned, pain itself can induce a stress response, including the horse showing both behavioral and neuroendocrine changes. Because of this, one should take in to account the amount of stress that is expressed during pain evaluation since the amount of stressors can affect the pain evaluation, but also in purpose to prevent the animal from developing distress (Gaynor & Muir, 2014).

Stress-induced analgesia

Although there are few studies relating Stress-Induced Analgesia (SIA) to horses one can assume results from human and human model studies, to have the same pathophysiological role in horses. It is already known that the emotional states in humans affects the individuals reactivity to a painful stimulus (Rhudy & Meagher, 2000), giving the emotional aspects of perception of pain a bigger role. The general idea of SIA is that an individual who are experiencing certain levels of stress is experiencing notably lower levels of pain during that moment. Butler & Finn (2009) describes SIA as “an in-built mammalian pain suppression response that occurs during or following exposure to a stressful or fearful stimulus”. This meaning that certain stressful events could decrease perception of pain within an animal. It is thought to be an evolutional trait, mainly for prey animals (Jennings *et al.*, 2014). Animals who were successful in concealing their pain during stressful events, such as when they were hunted, are thought to have better success in escaping, leading to that the animals who expressed pain during attack from predators were less successful in escaping, these animals in turn passed down this trait to its offspring. SIA can be divided in to two categories: conditioned and unconditioned SIA (Butler & Finn, 2009). The former describing pain which is concealed during a stressful event (e.g. fight or flight) and the latter describing analgesia during an event that the animal previously experienced as stressful, meaning that the analgesia is contingent. SIA is thought to be mediated through two types of pathways, one opioid and one non-opioid dependent pathway (Parikh *et al.*, 2011). Studies with transgenic mice who were given naloxone and naltrexone showed that they expressed less of the phenomenon (Ford & Finn, 2008). Furthermore, mice who completely lacked certain endogenous opioids did not express SIA at all, suggesting that at least part of the process is opioid-mediated. The other proposed system for downregulating pain is the endogenous cannabinoid system. Studies has shown elevated levels of endocannabinoids in mice expressing SIA (Ford & Finn, 2008).

Stress-induced hyperalgesia

As opposed to SIA where stress lowers the perception of pain, another type of stress can instead increase the amount of perceived pain within the individual. This phenomenon is called Stress-Induced Hyperalgesia (SIH). Whereas SIA more is an expression from an immediate or acute stressor, SIH instead is expressed when the animal is subjected to chronic or reoccurring stress (Jennings *et al.*, 2014). There are several methods for describing the possible pathways for SIH, including forced swim test, cold stress, immobilization or maternal separation to mention a few, most of the studies refer to a mediation through opioids, endocannabinoids and neuropeptides (Jennings *et al.*, 2014). Here it is important to note the differences between the different types of stress. As mentioned previously, stress can be defined to include both anxiety and fear. Where SIA is induced during a direct threat (fight or flight response), SIH is induced when the animals experiencing anxiety (Wagner, 2010). Wagner (2010) discusses that it, from an evolutional standpoint, makes natural sense that an animal would desensitize when fleeing from a direct threat as opposed to an animal who are anxious about an unknown threat who instead would be further sensitized. Therefore from a theoretical standpoint, there are novel evidence for the conclusion that fear reduces pain and anxiety enhances pain (Wagner, 2010).

Evaluation through observation of behaviours

Today, there is a paradigm shift towards incorporating behavioral measurements into the clinical evaluation of the horse. Post-operative pain is one field where a lot of behavior has been added in to better determine whether or not the horse is in pain (Price *et al.*, 2003; Pritchett *et al.*, 2003; Wagner, 2010). There are plenty of studies discussion potential behavioral indicators for stress, mainly of focus to assess animal welfare, several of which addresses coping mechanisms during stress (Budzyńska, 2014). Young *et al.*, (2012) has evaluated behavioral indicators for stress and has underpinned these indicators with physiological indicators (in this case heart rate and salivary cortisol levels). For low stress these values corresponded with (including, but not limited to) pacing, tail swishing, occasional weaving behavior, defecation, repetitive head movements and approach to potential stressors. When experiencing higher grades of stress, the horses showed a higher grade of stereotypical behaviors, more attention to the stressors and also showed a raised tail, nostril flares, muscular tension and snorting. This was in addition to previously mentioned behavior since there was an overlap of these behaviors. Furthermore, it has been shown, aside from stressed horses being more vigilant and spending less time eating, that horses who experience long term stress are more prone to develop stereotypic behaviors such as cribbing or weaving (Visser *et al.*, 2008). Aside from traditional ethological studies of stress behaviors, Ijichi *et al.*, (2013) has determined that a personality test performed on horses are able to predict certain behaviors based on the owners own assessment of the horse. In this study, horses who were categorized in certain groups based on the subjective predictions, very well determined how horses would react to visual stimuli and how well they recovered from this reaction. This could be a useful tool since behavioral studies of great magnitude takes time. Subjective assessment may be a promising replacement when full ethograms takes too much time. Ethograms have long been used in behavioral studies in order to evaluate horses' behaviors. It is a method widely used and can be applied to several sciences. Most recently ethograms have had a big role when evaluating welfare of the ridden horse, being a valid method for this type of evaluation (Hall & Heleski, 2017).

The Equine Facial Action Coding System

The Facial Action Coding System (FACS) is an method which, can describe all possible facial expressions in a human with the use of certain Action Units (AU) and Action Descriptors (AD) (Cohn *et al.*, 2006). These AUs and ADs are all produced by a contraction of a certain muscle in the face, which makes it an anatomically based system, leaving less space for subjective judgements (Wathan *et al.*, 2015). It is also seems to be a reliable tool, since people with no earlier experience of this type of reading were able to use the FACS-system with high agreement between coders. FACS has been adapted to use in several animal species, for example orangutans (Caeiro *et al.*, 2013), cats (Caeiro *et al.*, 2017) or dogs (Waller *et al.*, 2013). Developing an adaptation to use on horses were made in 2015 using dissection of underlying facial musculature in context with filming of natural occurring facial expressions of the horse (Wathan *et al.*, 2015). This proved different since the anatomy of the horse is different from other animals previously studied. This study resulted in a methodology called EquiFACS, the Equine FACS (table 2). EquiFACS can be classified as a type of ethogram entirely based on muscle anatomy. This is different from other ethograms that make use of grimaces, since these could reflect changes in other tissues.

Evaluation of pain using scales of facial expressions

There are at the present at least two ethograms that evaluates pain based only on facial expressions, the Horse Grimace Scale (HGS) (Dalla Costa *et al.*, 2014) and the Equine Pain Face (Gleerup *et al.*, 2015). The use of facial expressions is a promising tool for evaluating pain, especially when gross pain behaviours are absent. The methodology in these two studies however differed a lot. The HGS was developed using measurement of horses observed in a clinical environment before and after castration, which could leave uncertainties regarding medication and anaesthesia. The equine pain face was developed using blinded videos of horses with experimentally induced pain. Despite differences in pain type, duration and biases from medication and emotional states these two studies observed similar changes in facial expressions. Both scales found tension in the muscles above the eye, as well as the facial muscles laterally on the face (e.g. *m. masseter*). They also showed dilated nostrils and tension in the lips, making the muzzle change shape. However, the studies differed on some notes. The HGS makes a description of an orbital tightening and stiffly backwards ears while Gleerup *et. al*., (2015) instead found a more open eye and intense stare, along with a relaxation of the ear adductors. None of the studies addressed the possibility of emotional states affecting the pain evaluation. Dalla Costa *et al.*, (2017) researched the topic and found that most emotional states did little effect for the pain scoring, all emotional states except from fear. This is suggesting that stressful situations can affect pain scoring using these scales. Because pain probably induces fear in many instances and stress itself might produce some facial expressions, the lack of studies describing changes in facial expressions during stress induced by other causes than pain, is an apparent weakness of using facial expressions for the recognition of pain.

Table 1. *Summary of all codes in EquiFACS (Wathan et al., 2015).*

AU101 *Inner brow raiser*	**EAD104** *Ear rotator*
AU143 *Eye closure*	**AD19** *Tongue show*
AU145 *Blink*	**AD29** *Jaw thrust*
AU47 *Half Blink*	**AD30** *Jaw sideways*
AU5 *Upper lid raiser*	**AD133** *Blow*
AD1 *Eye white increase*	**AD38** *Nostril dilator*
AU10 *Upper lip raiser*	**AD50** *Vocalization*
AU12 *Lip corner puller*	**AD76** *Yawning*
AU113 *Sharp lip puller*	**AD80** *Swallow*
AUH13 *Nostril lift*	**AD81** *Chewing*
AU16 *Lower lip depressor*	**AD84** *Head shake side to side*
AD160 *Lower lip relax*	**AD85** *Head nod up and down*
AU17 *Chin raiser*	**AD86** *Grooming*
AU18 *Lip pucker*	**AD87** *Ear shake*
AU122 *Upper lip curl*	**AD51** *Head turn left*
AU24 *Lip presser*	**AD52** *Head turn right*
AU25 *Lips part*	**AD53** *Head up*
AU26 *Jaw drop*	**AD54** *Head down*
AU27 *Mouth stretch*	**AD55** *Head tilt left*
EAD101 *Ears forward*	**AD56** *Head tilt right*
EAD102 *Ear adductor*	**AD57** *Nose forward*
EAD103 *Ear flattener*	**AD58** *Nose back*

MATERIAL AND METHODS

Aim and hypotheses

The aim of this study was to investigate the changes in facial expressions during short term emotional stress in horses without pain and to compare these two with previously published changes of facial expressions during pain. To pursue this the objectives were:

1. Video record faces of healthy horses before and after subjected to stress
2. Obtain and use heart rate as a measure for stress and to obtain stress susceptibility of the horses subjected to stress
3. Annotate the changes in facial expressions before and during the stressful event using EquiFACS
4. Describe the characteristic changes of the horses experiencing stress
5. Briefly compare these changes to previously published facial changes in horses subjected to pain.

The hypotheses were that horses express facial expressions during stress that can be annotated by EquiFACS and that these expressions are similar to those of the equine pain face.

Study design

This study is an observational semi-randomized pilot project to determine whether the horse expresses stress through facial expressions. To study stress, two certain requirements need to be met. The animals must be well adapted to their base-line environment and the sampling must be minimally invasive in order to not have the sampling procedure affect the results (Novak *et al.*, 2013). To create a stressful situation for the horses with minimal intervention by observers, the study aimed to use transporting as the mean to have the horses stressed.

The horses were recorded during one hour in their own stable before being loaded in their transport. Potential stressful factors in the environment were minimized by letting the horse have at least one of their stablemate present in the stable at the time of filming. No change in the horse's routine were made when filming the base-line. No observers were present near the horse when filming, however people could be present in the stable at the time of filming. These people were usually there and it was determined that they should have little effect on the horses stress-levels since the horses were used to having people walking around them. After one hour of base-line recording the horses were taken to their carrier and loaded into the transport. The cameras and lighting were already mounted in the transport when the horse was loaded into it. The transport was driven on roads for between 10-20 minutes in order to be able to record enough suited video material.

Since no invasive methods were used to evaluate stress and since the horses was not exposed to anything out of their ordinary handling, no ethical permission was needed. Written consensus from the owners were obtained (see Appendix B).

Study population

In total, six horses were included in this study. The horses were owned by the department of Clinical Sciences, SLU except for two, who were privately owned. The latter were recruited through personal contacts. To ensure that the horses were healthy its owner or caregiver were given a questionnaire (see Appendix) regarding the horses' physical status and eventual medication. Since the aim of the study is to determine stress, only horses who were assessed as healthy by the owners were included.

Table 2. *List of horses included in the study.*

Horse no.	Age	Sex	Breed
1	22 years	Mare	Warmblood, trotter
2	20 years	Mare	Warmblood, trotter
3	5 years	Mare	Swedish warmblood
4	7 years	Gelding	Thoroughbred
5	17 years	Gelding	Warmblood, trotter
6	9 years	Gelding	Warmblood, trotter

Inclusion criteria

Following criteria were used to include the horse in the study population. One or several criteria could be used:

1. Known distress when transported by the owner
2. Never been transported before
3. The horse was sweaty when exiting the transport and this did not occur because of high temperature.

Heart rate measurement

To further ensure that the horse on camera is filmed during a stressful event, measurement of heart rate was done on the horses both in the stable and in the transport. To be able to measure heart rate constantly and without interference (by observers) with the horses, two remotely controlled pulse bands (Polar Wear Link) along with one controlling unit (Polar RS5800CX Watch) respectively. The pulse bands were attached on the horse with the girth just behind the withers with the Wear Link placed on the skin over the cardiac silhouette on the left-hand side of the horse. The lap-function on the control-unit were used to mark when the horse was taken to the trailer and when it was taken off it. The heart rate data were analyzed in Polar Protrainer 5 Equine Edition. The data was exported to Microsoft Excel for statistical analysis. The mean heart rate and increase was calculated. Significance was calculated using a paired two-way t-test on mean values with the significance level set at $p<0.05$.

Figure 2. *Placement of the Polar Wear Link.*

Video filming

The horses were video filmed for evaluation in order to minimize interaction with observer. During recording, GoPro Hero 3+ Silver Edition were used, both in the transport and in the stable. In the stable the cameras were mounted approximately at withers-lever in a corner were the horse were most prone to stand. In some instances, two cameras were used for the same horse to make sure that enough good quality base-line material were available. In the transport, the cameras were mounted at front with an angle of approximately 45 degrees from the horse's median plane. The horses name was presented for the camera at start of filming to ensure pairing right films with the right horse.

Handling of videos and selection of sections for annotation

The videos were edited in Camtasia 9. The first minute of the base-line films were cut out to ensure that the person who set up the camera would have left the stable. The films were after that evaluated using an ethogram containing gross behaviors and head movements. Three-minute segments were analyzed from each horse. These were selected by using a random number generator to pick the starting point of the three-minute segment. Afterwards, all thirty-second segments were the horses face were visible and codable by EquiFACS were cut out and given a number. Using a random number generator, one thirty-second segment per horse were selected and analyzed. An ethogram according to EquiFACS was created, excluding head movements and gross behaviors which were analyzed in the other film.

Videos from the transport were cut in a similar way. The first minute were cut out to ensure that no disruption were in the movie. Using a random number generator, the starting point for a three-minute segment were selected and cut out for each horse. These segments were later analyzed using an ethogram containing all EquiFACS ADs and AUs (including head movements and gross behaviors). Segments of the clip which were facial expressions were not visible were assigned the code VC74 (not possible to annotate), however head movements and gross behaviors were still coded during these segments as they were still visible in all clips.

Analysis of data

Heart rate-data were exported to Microsoft Excel ®. Each measurement was approximately measured three seconds apart by the pulse-clock. Mean values were calculated using Excel. These mean values were then associated to the time period were the horse was standing in the stable or being transported. These data were then used for analysis. A two-way paired t-test was used to calculate significance.

The software ELAN was used to mark the number of times a certain Action Unit or Action Descriptor were present in the clips. All evaluations were done by a certified EquiFACS reader to secure the validity of the scoring. To perform simple descriptive statistic of the sample population, and to create tables and diagrams of the collected data, Microsoft Excel were used.

The number of AUs and ADs present in the clips were plotted in table 7 and appendix A. The most frequent AUs and ADs were selected. The frequency of these actions units during transportation was calculated using the time of the clip and the percentage of the clip that was codable. If baseline equaled zero a one-way paired t-test on mean values were used (since the frequency could not be negative values), otherwise a paired two-way test for mean values was used. Contingency tables were made presenting the number of horses which had an increase in respectively action unit or action descriptor. Significance was calculated using Fisher's exact test. Significance level was set at $p<0.05$.

RESULTS

A total of 6 horses were recorded. During filming of one of the horses the camera stopped working after 3 minutes, therefore no randomisation process was performed for this horse but the whole film obtained were analysed. On one horse (no 6) the pulse-clock lost contact during transport. These measurements were excluded from the analysis.

Heart-rate during transportation

Measurements of heart rate showed that five of the six horses (horse number 6 could not be evaluated) had an increase in heart rate when being loaded in to the transport. The mean heart rate in the stable varied between 33 and 57 beats per minute while the same horses experienced an increase in heart rate with a between 53 and 91 beats per minute. The mean increase of heart rate during transport were 161.4 percent of the baseline value. The increase was significant in all five horses. The results are presented in table 3.

Table 3. *Mean heart rate (HR) for baseline (B) and transports (T) and increase (percent of baseline-value) for each horse.*

Horse no	**Mean HR_B**	**Mean HR_T**	**Increase**	**p**
1	43	74	172 %	<0.001
2	33	61	185 %	<0.001
3	46	74	161 %	<0.001
4	57	91	160 %	<0.001
5	41	53	129 %	<0.001

Changes in FACS during transportation

All AUs and ADs were recorded and are presented in table 5. If one AU or AD were not present at all, it was not included in the table. This table was then used to create the figures in appendix B which illustrates the number of times a certain AU or AD were present in each horse. These tables suggested that the Action Unit numbered 5 (AU5) were present in a higher grade when the horse is being transported as well as the Action Descriptors number 19 and 38. Therefore, these facial units were selected for further analysis. The percentage of each film where the sections were not possible to obtain annotations from is presented in table 4.

Table 4. *Percentage of the transportation-films which were uncodable (VC74)*

Horse no	**Percentage VC74**
1	51%
2	53%
3	65%
4	72%
5	37%
6	79%

Table 5. *Total occurrences of recorded AU's and AD's during baseline (B) and transport (T)*

Horse	**1_B**	**1_T**	**2_B**	**2_T**	**3_B**	**3_T**	**4_B**	**4_T**	**5_B**	**5_T**	**6_B**	**6_T**
AD1	0	0	0	4	1	1	0	12	1	3	2	1
AU101	0	2	0	0	0	0	0	0	0	1	0	1
AU145	1	22	6	11	2	12	9	20	2	4	2	10
AU47	2	12	1	6	1	3	0	5	0	6	0	0
AU5	0	11	0	12	0	2	0	25	0	16	0	6
AU10	1	0	0	1	0	0	0	0	1	0	0	1
AU113	0	0	0	0	0	0	0	0	0	1	0	0
AU12	0	0	0	0	0	0	0	0	0	1	0	0
AU16	0	1	1	0	0	1	0	0	0	11	0	1
AU18	0	0	0	0	0	0	0	5	0	0	0	0
AU25	0	5	1	16	0	0	0	13	0	7	0	3
AUH13	0	1	0	3	0	0	0	7	0	0	0	0
AD133	0	1	0	0	0	0	0	0	0	0	0	0
AD19	0	4	1	17	0	0	0	13	0	1	0	15
AD38	0	13	0	17	0	21	1	14	0	4	1	14
AD81	0	0	7	0	2	2	0	0	1	0	4	0
AD84	0	0	0	0	0	1	1	5	0	0	0	0
AD85	0	0	0	0	0	0	0	10	0	1	0	1
EAD101	1	27	2	26	3	25	3	17	2	43	2	11
EAD104	1	24	2	27	4	15	2	21	2	40	1	10
AD51	2	1	2	13	5	6	13	21	0	3	1	7
AD52	4	4	1	5	6	11	0	10	3	4	0	19
AD53	0	1	0	1	0	0	0	0	0	0	1	1
AD54	7	6	3	4	4	1	3	13	0	9	1	13
AD57	0	2	0	2	0	0	0	1	0	1	2	6
AD58	0	0	0	0	0	0	0	0	1	2	0	0

The selected codes are presented in table 6. All three types of codes increased in frequency during transportation but not individually for each horse. AD25 (lips part) were not included since it was coded mainly when tongue show (AD19) was coded.

Table 6. *Frequency of selected codes during baseline (B) and transportation (T)*

Horse	$AU5_B$	$AU5_T$	$AD19_B$	$AD19_T$	$AD38_B$	$AD38_T$
1	0/s	0.12/s	0/s	0.04/s	0/s	0.15/s
2	0/s	0.14/s	0.03/s	0.20/s	0/s	0.20/s
3	0/s	0.03/s	0/s	0/s	0/s	0.33/s
4	0/s	0.49/s	0/s	0.26/s	0.03/s	0.28/s
5	0/s	0.14/s	0/s	0.01/s	0/s	0.04/s
6	0/s	0.15/s	0/s	0.34/s	0.03/s	0.32/s
Mean increase		*0.18/s* *p=0.02*		*0.14/s* *p=0.06*		*0.21/s* *p=0.005*

Action Unit 5 and Action Descriptors 19 and 38 where further tested for significance using contingency tables (Table 7). The tables show on a population level how many horses who had an increase of each code during transport. With $p<0.05$ for AU5 and AD38 these changes in the horses' facial expressions were significant, as for AD19, $p>0.05$ and were therefore not significant.

Recorded movements during transportation

The horses were scored with the number of movements as in compliance with EquiFACS, this including movements of the head and ears as well as the frequency of blinks and "half-blinks". The hypothesis was that horses experienced a more outward behaviour when exposed to emotional stress, which when regarding head movements would express itself as a higher frequency of movements. For ears and blinks this would also mean an increase. No special regards were taken to the direction of the movement when analysing head and ear movements, as the frequency of movements were of interest. The mean frequency of ear movements (EAD101 and EAD104) increased during transportation, as well as the frequency of blinks (AU145 and AU47) and head movements (AD51-58). The results are presented in table 8. The two-way paired t-test for mean values showed that the increase was statistically significant.

Table 7. *Contingency tables containing the number of horses where the frequency of certain codes increased during transportation.*

	AU5+	**AU5-**	***Total***
Baseline	*0*	*6*	***6***
Transport	*6*	*0*	***6***
Total	**6**	**6**	***12***
			p=0,0022

	AD19+	**AD19-**	***Total***
Baseline	*1*	*5*	***6***
Transport	*5*	*1*	***6***
Total	**6**	**6**	***12***
			p=0,0801

	AD38+	**AD38-**	***Total***
Baseline	*0*	*6*	***6***
Transport	*6*	*0*	***6***
Total	6	6	*12*
			p=0,0022

Table 8. *Mean frequency of movements during baseline and transport (movement/second)*

	Baseline	***Transport***	***p***
Ear movements	*0.13/s (SD = 0.07)*	*0.62/s (SD = 0.12)*	*<0.001*
Blinks and half-blinks	*0.14/s (SD = 0.09)*	*0.27/s (SD = 0.13)*	*0.043*
Head movements	*0.05/s (SD = 0.03)*	*0.15/s (SD = 0.06)*	*0.028*

DISCUSSION

Stress as shown through facial expressions

When the horses were exposed to the stressor it became clear that they exhibited some tension in certain facial muscles. Most apparent were the show of AU5 (upper lid raiser) and AD38 (nostril dilator). The presence of these two were also the only ones who were significant when measured quantitative. One could argue that AD19 (tongue show) would be of significance since the behaviour were present in all horses but one and in greater frequency than other non-stress associated codes. Therefore, this Action Descriptor is included in the report since, with a larger population, it could possibly be shown to be significant. The results show a tendency that there are changes in facial expressions when a horse is exposed to emotional stress. During baseline some of these expressions were visible in the horse but in less extent. For example, some, horses dilated its nostrils in a non-stressful situation. However, during transporting the increased frequency of this Action Descriptor were of importance since this showed a difference in behaviour for that particular horse.

Since it has been shown before that facial expressions are responses to emotional stimuli in animals (Cátia *et al.*, 2017) it is not that surprising that horses express stress through facial expressions. When a horse is experiencing fear or distress, gross behaviours (e.g. tail swishing or pawing) has been studied well but little attention has been brought towards the facial expressions in combination with the emotional aspect. The facial expressions that has been concluded in this study could have several purposes. Firstly, facial expressions could display emotions towards conspecifics. Secondly, tension in these facial muscles could have an evolutionary purpose. Since the horse is a flight-animal, raising of the upper eyelid could have a part in scanning the surroundings for danger when stressed. Similarly, dilatation of the nostrils may have a part in preparing the animal for flight. Interestingly, AU5 and AD38 are also present when humans are experiencing fear (Cátia *et al.*, 2017). Since this exposure to a stressor would evoke nostril dilation and upper lid raiser in humans it would support the hypothesis that the transports in fact are a stressor for these horses.

General behaviour during stress

During transportation, certain traits became more distinct. In general, the horses exhibited a lot more movements, especially when comparing to the behaviour in the stable. Blinking, half-blinking and ear movements tend to be naturally occurrent during non-stressful situations and are also descriptors that contain physiological function (e.g. blink). Once again, it is the increased frequency of these behaviours that could potentially indicate stress.

One should however keep in mind that ear- and head movements are normal behaviours when the horse is experiencing a lot of new impressions, and need not for that sake be a result of only stress. Exposure to new environments but lack of stress could potentially show an increase in frequency for these codes as well. With that said, these gross behaviours have a lot of support when looking in to other studies. As mentioned earlier in this report, horses who experience stress often express contact-seeking and extrovert behaviour, e.g. tail swishing, head bobbing or pacing. This fits in rather well with the results in this report. More extrovert behaviour would consequently give a higher frequency of movements. As for the frequency of eye blinks, earlier studies have shown an increase of blinks (Reid *et al.*, 2017) during stressful situations which coincides with the findings here.

As mentioned earlier, stress is to maintain homeostasis after the body is threatened. Reasonably should coping behaviours help protecting the body from an evolutional standpoint. Moving their heads and ear often should help to detect enemies faster since the horse is more vigilant and aware of its surroundings. Frequent blinks should help keeping the visions clear. What is interesting to note is that these types of behaviours seem almost borderline with stereotypical behaviour. Asserting further that exposure to chronic stress could cause a flashover behaviour.

Stress as an interference when interpreting pain

The results in this thesis suggests, together with studies presented in the literature review, that stress induces a more outward response in the horse, possible as some sort of displacement behaviour. Since stress has been known to cause these types of behaviours, as well as stereotypical behaviours (Young *et al.*, 2012), it should not come as a surprise that these behaviours are shown in the facial expressions as well.. Horse do express a wide variety of behaviours when in pain. A horse with colic show behaviours such as pawing, restlessness and movement of the head towards the painful area, while horses in chronic mild pain showed more introvert behaviour which is similar to depression (Gleerup & Lindegaard, 2016). It cannot be concluded whether or not this is strictly pain-related behaviour or if a great amount of pain induces stress that presents itself this way. Since stress and pain are similar in expression and physiology, it is not impossible to think that stress potentially could cancel out pain related behaviours. This theory is further supported by the description of stress-induced analgesia which were processed earlier in this report.

One could also argue that the facial expressions could be cancelled out as well. With the support of the results in this thesis, stress could induce tension in facial muscles that potentially could aggravate, if not completely mask, assessments of pain using facial expressions. The tension of orbital muscles with the prominent tension in *m. levator anguli occuli medialis* (AU101 in EquiFACS) could be harder to read if AU5 is present in a stressed horse. It would also be harder to determine if the tension in orbital muscles is dependent of stress or pain. The cone-shaped nostril would be harder, if not impossible to see if AD38 (nostril dilator) is present. The tense muzzle could be cancelled out by excessive moving and part of the lips and movement of the tongue. Dilated nostrils, which is a prominent part in both the equine pain face and HGS, is also present during stress (AD38) making this part of the pain face harder to evaluate. Lowered, asymmetrical ears (pain face) or stiffly backwards ears (HGS) would be harder to detect if there are a lot of movement of the ears. This could indicate a possible need to know about possible stressors for the horse when evaluating pain using facial expressions.

Strengths and limitations with the study

When evaluating this study, one question in particular need to be answered: Is it in fact stress that has been measured? Several aspects need to be taken into account. To begin with, heart rate was measured during both baseline-filming and filming during transportation. As mentioned earlier, heart rate may be a valid parameter to evaluate stress. However, one must take into account that heart rate can be affected by many factors e.g. age, condition, size etcetera and therefore it cannot be used as only instrument of measuring stress. Even though several of the horses were of different breeds, ages and sexes there is still a too small of a population to really cancel out these factors. In this study, no further measurements were made on the horses since invasive methods could potentially interfere with result. Instead inclusion criteria were set so that the horses included could be presumed to be stressed during the exposure to the stressor. This is a highly subjective method, and this should be taken into account when interpreting the results.

Another important aspect when evaluating stress and its potential effect on pain evaluation is making sure the horses included were not in pain at the moment of filming. Using owner questionnaires is a simple yet reliable method since the owner often knows its own horse and its earlier ailments but is also a subjective, and in some instances, an unreliable method. However, there is also the possibility of the owner not noticing pain in the horse. This means that horses can not only be included based of the questionnaire, but further assurance is needed. In this instance, the baseline filming was used. If the horse showed no signs of facial codes suggesting a “pain face” (Gleerup *et al.*, 2015), the horse, together with an approved questionnaire, could be assumed to be healthy and without pain.

In this study the annotation of the selected sets of AUs and ADs were analysed in a software which allowed frame-by-frame analysis. This were of benefit since it generated a reliable identification. Frame-by-frame analysis is also recommended when using EquiFACS (Wathan *et al.*, 2015), however, it is very time consuming. Approximately 2 hours per 30 second-clip. The use of film instead of still images were of importance, since the use of still images is not possible when coding certain action descriptors e.g. blinks or head movements. It is also important to note that certain actions units/descriptors are not present in the horse at all times. Horses are curious animal and objects in the environment, sound and smells may temporarily hide certain facial traits. The strengths of measuring the frequency of certain facial expressions over time in a video clip is that there are lower chances of these distractions affecting the result.

The video-recording and the heart-rate instrumentation could be done within two hours per horse, after routine was obtained. However, this was dependent on the willingness of the horse in question.

A total of six horses were included in this study. This sample size can be criticized for being low, and not suited for statistical evaluation. However, this study aimed to highlight possible hypotheses for further studies on emotional stress and test suited methodologies. Also, the sample population was very narrow since two thirds of the population came from the same place, as well as same breed. With a bigger sample size, one could possibly draw further conclusions regarding facial expressions during emotional stress, generating possibly more significant results. Also, repeated significance testing could generate a higher risk of false significant values. The study therefore focuses on the quality of the measurements rather than quantity. However, since the differences in presences of action units were statistically insignificant, rigid conclusions regarding the effect of stress on the reading of pain face cannot be drawn.

Future uses

It is well known that when a cat, one of the more easily stressed animals at a companion animal clinic, is examined out of its home, its heart rate and temperature rises almost immediately, only from the car ride in. Dog owners are requested to count respiratory rate at home since stress in the clinic affects the outcome and is therefore not trustworthy. Implications of stress are already treated in the small animal practice yet little regard is considered of the stress in the large animals that are treated on clinic. Potential use of a stress face could be used in clinical environments to assess stress, a procedure that could be of great benefit when evaluating either pain or clinical parameters when assessing horses.

Further research is also necessary to understand how emotional states affects the equine pain face. The equine pain face was researched during artificial situations without emotional stimuli (Wathan *et al.*, 2015). Thus, stress or other emotional states in the horse could be of big significance when dealing with pain evaluations using facial expressions, especially in a hospital or equal environment where the horses are subjected to a lot of stress. Example on research could include exposing the same horses for emotional stress and thereafter induce pain. On these horses one could more easily differentiate between stress and pain. One could also study horses who are believed to be in pain, suffering from painful conflictions. When the horses have been treated for pain the horses could be studied with regards to stress.

Studies of stress could also be of benefit, not only in a clinical environment, but also in environments were stress is occurring and needed to be identified. One example on such situations may be when the horse is used in sport. Identification of a stress face may be reliable tool to identify stress and ensure welfare for horses during competitions and other events where welfare potentially may be compromised.

CONCLUSION

The results in this pilot study shows a tendency towards that the horse do express certain traits that can be objectively registered using EquiFACS. These traits include a dilation of the nostrils, raising of the upper eyelid and frequent use of the tongue. Furthermore, the horse tends to act more outwards when exposed to stress which can be characterized by frequent ear movements, the horse blinking more than usual and also an increase in head movements. Expressions from a stressed individual could potentially cancel out behaviours otherwise related to pain in that horse. Therefore, further research, especially with a bigger population, could draw more conclusions regarding the interference with pain evaluation as well as finding and evaluating stress using behavioural methods. Further research should also involve a stratification of horses with respect to tendency of development of emotional stress. Experimental studies of facial expressions of pain should be performed in horse with and without experience of concurrent stress to study the effects of stress on the pain face.

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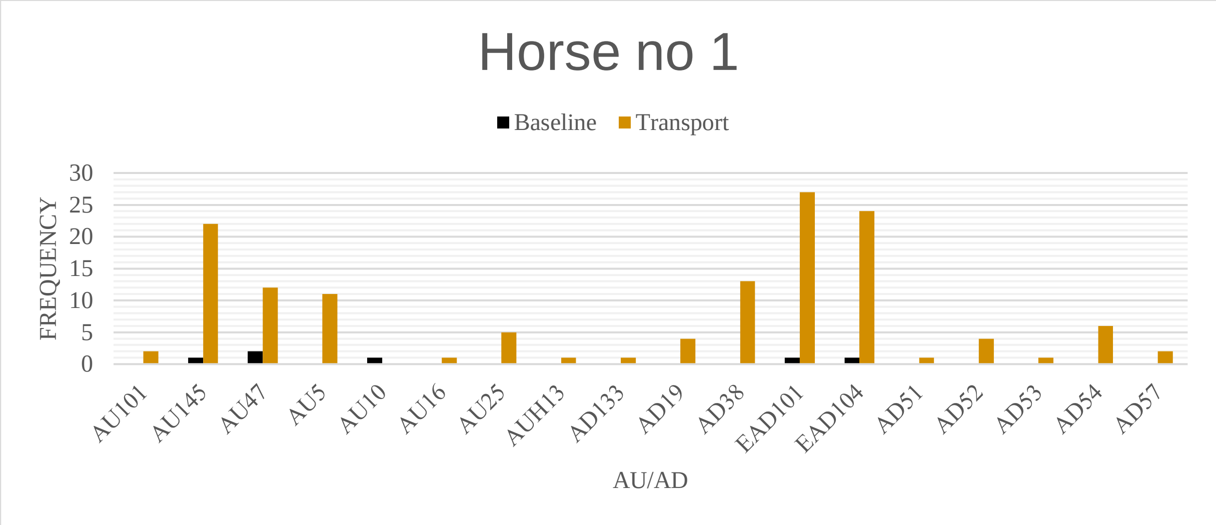
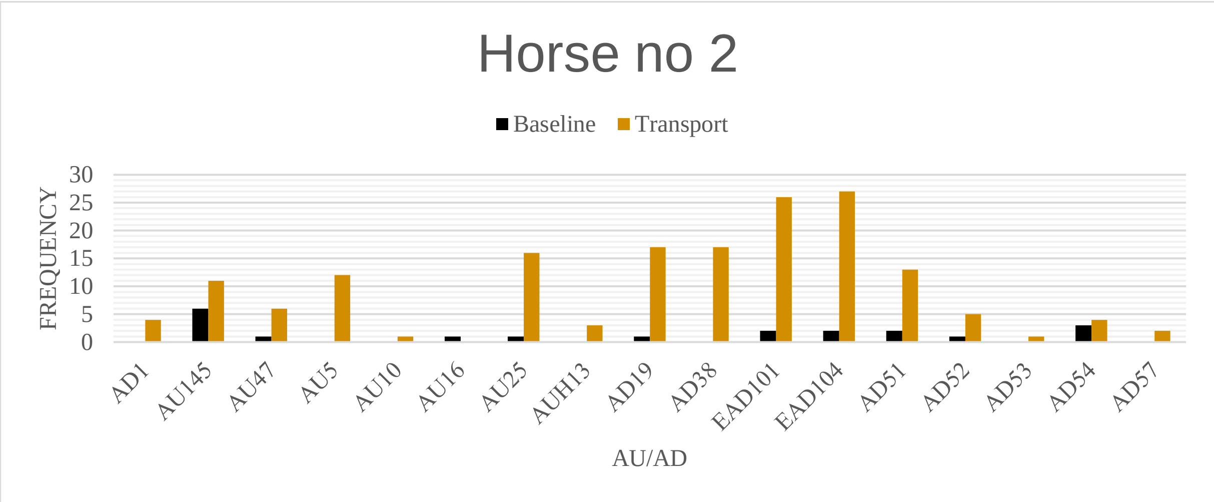
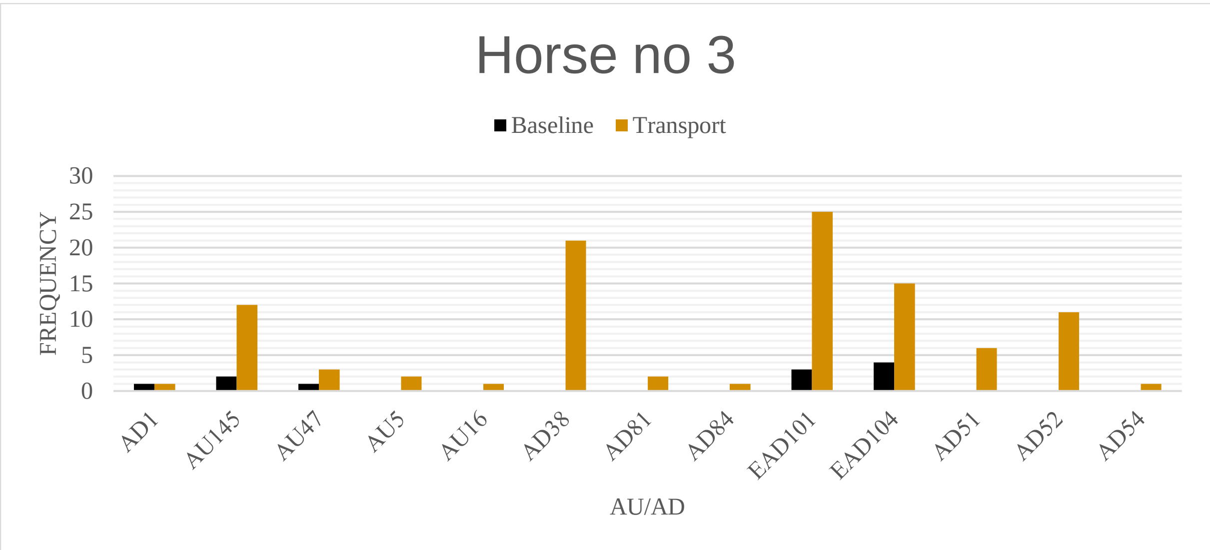
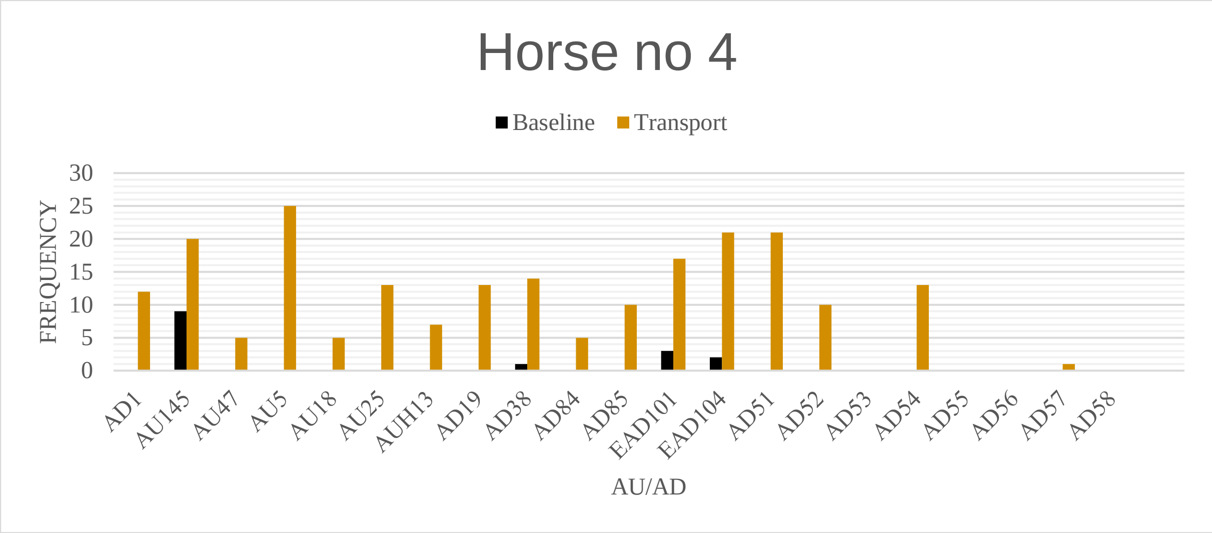
APPENDIX A: Supplementary figures

These figures were made using the data form table 7 to further illustrate the selection of appropriate Action Units and Action Descriptors for analysis.

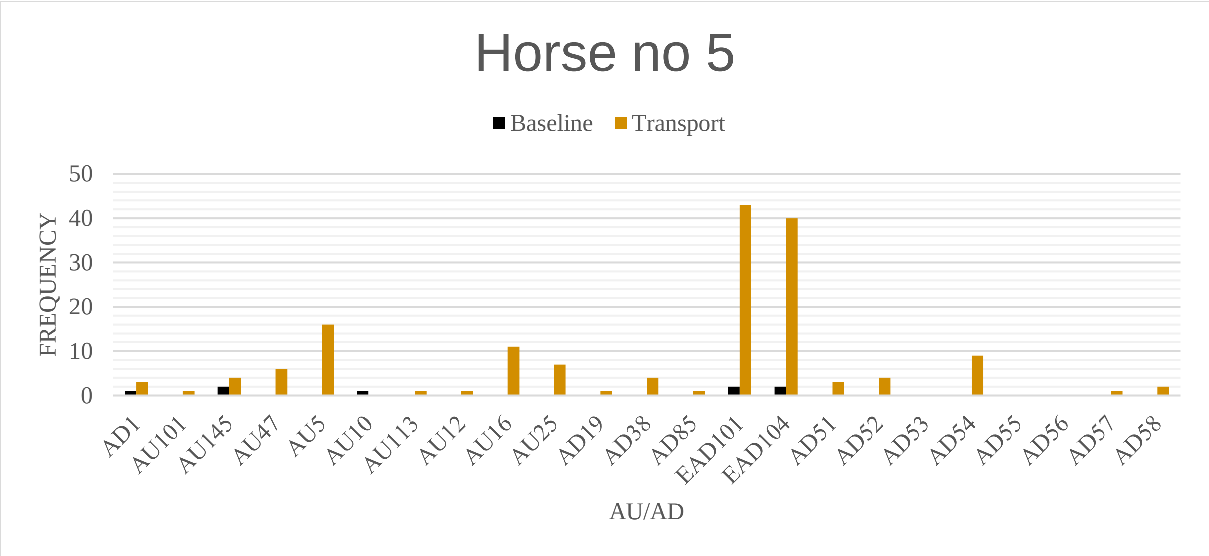




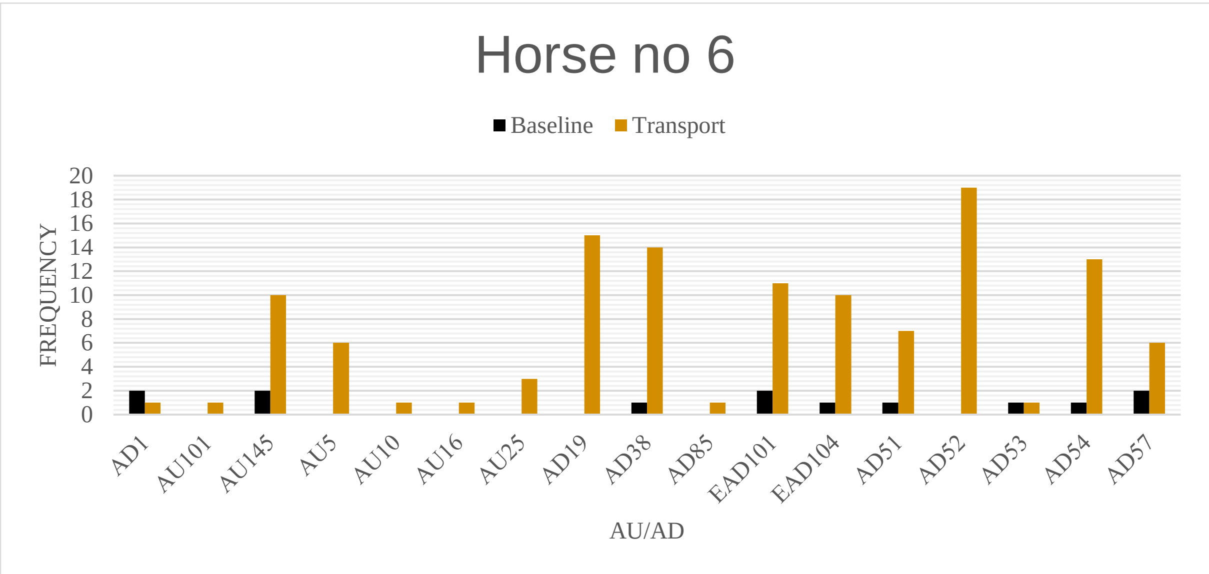




Horse no 4
Baseline
Transport
FREQUENCY
30
25
20
15
10
5
0
AD1
AU145
AU47
AU5
AU18
AU25
AUH13
AD19
AD38
AD84
AD85
EAD101
EAD104
AD51
AD52
AD53
AD54
AD55
AD56
AD57
AD58
AU/AD



Horse no 5
Baseline
Transport
FREQUENCY
50
40
30
20
10
0
AD1
AU101
AU145
AU47
AU5
AU10
AU113
AU12
AU16
AU25
AD19
AD38
AD85
EAD101
EAD104
AD51
AD52
AD53
AD54
AD55
AD56
AD57
AD58
AU/AD



Horse no 6
Baseline
Transport
FREQUENCY
20
18
16
14
12
10
8
6
4
2
0
AD1
AU101
AU145
AU5
AU10
AU16
AU25
AD19
AD38
AD85
EAD101
EAD104
AD51
AD52
AD53
AD54
AD57
AU/AD

APPENDIX B: Owner questionnaire and permission

Djurägarmedgivande

Under hösten 2017 kommer Johan Lundblad med Pia Haubro Andersen som handledare att undersöka huruvida kortvarig emotionell stress inverkar på hur vi uppfattar smärta hos hästen. Vi kommer i försöket använda oss av ett mätsystem för att kartlägga hur hästen använder sina ansiktsmuskler i samband med att den utsätts för stress. För att undersöka detta vill vi videofilma din häst i under en kort period (ca 20 min) under transport med släpvagn/hästtransport samt under en lite längre period inne i sin egen box/i lugn miljö. Din häst kommer inte ha ont under försöket utan endast visa upp via ansiktsmimiken ifall den inte gillar att åka transport.

Förutsättningar för att vi ska kunna använda filmen från din häst

Hästen ska vara frisk och får inte ha någon skada under tiden för försöket.

Finns det några nackdelar med att min häst deltar i studien?
Nej. Vi kommer endast montera kameror i transporten och i stallet. Kamerorna är stöttåliga och kan inte gå sönder. Vi kommer också sätta på ett band för att mäta hjärtfrekvensen.

Finns det några direkta fördelar med att min häst deltar i studien?
Nej. För att säkra giltigheten i studien kan vi inte ge dig några fördelar för att medverka. Din medverkan i studien kommer sannolikt att hjälpa oss förstå bättre hur hästar uttrycker stress men kommer inte att ge dig som djurägare någon enskild feedback om just din häst. Vi erbjuder inte finansiell ersättning för medverkan i studien.

Kommer mitt namn eller hästens namn synas i anslutning till studien?
Nej. Ditt namn och hästens namn kommer endast vara känt för den student som bearbetar filmerna. Alla svar på enkäter är konfidentiella.

Kontaktpersoner:
Johan Lundblad
Prof. Pia Haubro Andersen

Jag ger härmed försöket tillstånd att videofilma min enligt ovan. Jag tillåter även publicering av insamlat bildmaterial i följande kategorier.

☐ A) Webben

☐ B) Tryckt form, t.ex.

☐ C) I undervisning eller på konferenser

☐ Jag tillåter min hästs medverkan i studien men tillåter ingen publicering av insamlat bildmaterial.

Datum:_______________ Hästens namn: ____

Ägare/Ansvarig:________________________________Legitimation:_____________________

Frågeformulär för djurägare

Fråga 1: Har din häst behandlats av veterinär det senaste året?

☐ Ja ☐ Nej

Om ja, för vad?

Fråga 2: Har din häst någonsin blivit diagnosticerad med en sjukdom av kronisk karaktär t.ex. artros, fång etc.

☐ Ja ☐ Nej

Om ja, vilken?

Fråga 3: Upplever du idag att din häst är frisk och smärtfri?

☐ Ja ☐ Nej

Fråga 4: Har din häst det senaste året varit halt?

☐ Ja ☐ Nej

Fråga 5: Har din häst under det senaste året behandlats med smärtlindrande preparat?

☐ Ja ☐ Nej

Om ja, vilket?