Facial expression of pain in horses after colic surgery or castration
Clinical application and effect of analgesic treatment

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Facial expression of pain in horses after colic surgery or castration – clinical application and effect of analgesic treatment
En undersökning av hästens ansiktsuttryck vid smärta efter bukoperation eller kastration – klinisk applicerbarhet och effekt av smärtlindrande behandling

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SUMMARY

To ensure that an animal in pain receives the correct analgesic treatment pain assessment is of great importance. Recent research has shown that horses change their facial expression during pain, and shows a so called pain face. Using pain face as a pain assessment tool in horses is only sparsely validated but is of great interest since studies in humans have shown that it is less possible to hide a pain face. The aim of this study was to investigate the clinical application of the equine pain face as a pain assessment tool during clinically relevant conditions. The hypothesis was that facial expression of pain in postoperative horses would disappear or diminish after analgesic treatment. The secondary aim of this study was to investigate the performance of the equine pain face as a pain assessment tool in postoperative horses.

Eleven horses that underwent exploratory laparotomy due to colic and one horse that developed hemoperitoneum after castration were included in the study. The behaviour and face of the horses were filmed postoperatively at two occasions, before analgesic treatment and two hours after analgesic treatment. The randomised video films were shown to seven blinded observers, who pain scored each horse according to facial expression of pain (no pain face, pain face present or intense pain face) and a modified version of the Equine Pain Scale (EPS).

Facial expression of pain was observed in all of the twelve horses included in the study. However, no significant difference was seen in grading of facial expression of pain or the score of the modified EPS before and after analgesic treatment. This indicates that either the analgesic treatment was not always optimized or that there could be several other conditions that contributed to the changes in the face mimic. Fleiss kappa for inter-observer reliability of facial expression of pain was determined to 0.11 (slight agreement). The low kappa value is most likely due to the subjective grading of facial expression of pain, concurrent or intermittent lidocaine and butorphanol treatment and differences in observers’ ability to evaluate facial expression. Investigations of the equine pain face regarding the number of present facial action units that are needed and a weighting of the facial action units is of great interest in order to create a higher inter-observer reliability. Intra-observer reliability was determined to a mean value of 0.51 (moderate agreement). Furthermore, five out of seven observers had a kappa value above 0.5, suggesting that facial expression of pain could be a good supplementary pain assessment tool when a single person observes a horse over a time.

The score of the modified EPS was compared to the grading of facial expression of pain. The included horses were divided into three different groups on the basis of their total score of the modified EPS; group 1 (score of 0-3), group 2 (score of 4-7) and group 3 (score of 8-11). Sixty-three % of the horses in group 1 expressed a pain face and sixty % of the horses in group 2 expressed a pain face. Furthermore, hundred % of the horses in group 3 expressed a pain face. This study evaluated pain with a visceral component. Further studies regarding the specificity and sensitivity of facial expression of pain are required when complex pain types and medication are involved.

SAMMANFATTNING
För att kunna säkerställa att djur med smärta får en korrekt smärtbehandling är smärtbedömning särskilt viktigt. Nyligen publicerade studier visar att hästar uttrycker specifika ansiktsutttryck vid smärta, ett så kallat ”smärtansikte”. Användning av ansiktsutttryck vid smärtbedömning är ännu inte fullt validerat hos hästar, men det är mycket intressant eftersom studier på människor har visat att ett ”smärtansikte” inte kan döljas helt med viljan. Syftet med studien var att undersöka den kliniska applicerbarheten av hästens ”smärtansikte” som ett verktyg i smärtbedömning. Hypotesen var att ansiktsutttryck för smärta hos postoperativa hästar skulle försvinna eller avta efter smärtlindrande behandling. Studiens sekundära syfte var att undersöka pålitligheten och sensitiviteten av gradering av ansiktsutttryck för smärta hos postoperativa hästar.

Elva hästar som genomgick explorativ laparotomi på grund av kolik samt en häst som utvecklade hemoperitoneum efter kastation deltog i studien. Hästens beteende och ansikte filmades postoperativt vid två tillfällen, strax före och 2 timmar efter smärtlindrande behandling. Filmerna randomiserades och visades för 7 blindade observatörer, vilka hästens beteende och ansikte filmades postoperativt vid två tillfällen, strax före och 2 timmar efter smärtlindrande behandling. Filmerna randomiserades och visades för 7 blindade observatörer, vilka Hästens beteende och ansikte filmades postoperativt vid två tillfällen, strax före och 2 timmar efter smärtlindrande behandling. Filmerna randomiserades och visades för 7 blindade observatörer, vilka Hästens beteende och ansikte filmades postoperativt vid två tillfällen, strax före och 2 timmar efter smärtlindrande behandling. Filmerna randomiserades och visades för 7 blindade observatörer, vilka Hästens beteende och ansikte filmades postoperativt vid två tillfällen, strax före och 2 timmar efter smärtlindrande behandling. Filmerna randomiserades och visades för 7 blindade observatörer, vilka Hästens beteende och ansikte filmades postoperativt vid två tillfällen, strax före och 2 timmar efter smärtlindrande behandling. Filmerna randomiserades och visades för 7 blindade observatörer, vilka Hästens beteende och ansikte filmades postoperativt vid två tillfällen, strax före och 2 timmar efter smärtlindrande behandling. Filmerna randomiserades och visades för 7 blindade observatörer, vilka Hästens beteende och ansikte filmades postoperativt vid två tillfällen, strax före och 2 timmar efter smärtlindrande behandling. Filmerna randomiserades och visades för 7 blindade observatörer, vilka Hästens beteende och ansikte filmades postoperativt vid två tillfällen, strax före och 2 timmar efter smärtlindrande behandling. Filmerna randomiserades och visades för 7 blindade observatörer, vilka Hästens beteende och ansikte filmades postoperativt nidingsbet och butorfanol och att det finns skillander i observatörers förmåga att bedöma ansiktsutttryck. För att höja pålitligheten mellan observatörer är det viktigt att undersöka hur många ansiktsdrag för smärta som märkte ses för att säga att en häst har ont, samt vilka ansiktsutttryck som är störst betydelse för att indikera smärta hos hästar. Medelvärden för pålitligheten inom varje observatör bestämdes till 0.51 (måttlig överensstämmelse), där 5 av 7 observatörer hade ett kappa-värde över 0.5. Detta indikerar att hästens ”smärtansikte” kan vara ett bra komplementärt verktyg då en enskild individ vill följa en hästs smärtstånd över en tid.

En jämförelse gjordes mellan graderingen av ansiktsutttryck för smärta och poäng från den modifierade versionen av EPS. Häftarna delades in i tre olika grupper med hänsyn till deras poäng från den modifierade versionen av EPS; grupp 1 (poäng 0-3), grupp 2 (poäng 4-7) och grupp 3 (poäng 8-11). 63% av hästarna i grupp 1 uttryckte ett ”smärtansikte” och 60% av hästarna i grupp 2 uttryckte ett ”smärtansikte”. Vidare uttryckte 100% av hästarna i grupp 3 ett ”smärtansikte”. Denna studien undersöker smärta som inkluderar viscerala komponenter. Ytterligare studier om specifict och sensitiviteten gällande hästens ansiktsutttryck för smärta vid komplexa smärttyper och medicinering krävs.

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INTRODUCTION

Animal pain is defined as “an aversive sensory experience caused by actual or potential injury that elicits protective motor and vegetative reactions, results in learned avoidance behaviour, and may modify species specific behaviour, including social behaviours” (Zimmermann, 1986: see Rutherford, 2002 p. 31). Minimizing pain is of great animal ethical interest. Moreover, studies have shown that early and forceful pain control improve recovery, reduce catabolism and shorten the hospital stay (Yardeni et al., 2007; McGuire et al., 2006; Shavit et al., 2005; Sellon et al., 2004). Therefore it is important for animals in pain to receive a good analgesic treatment, with correct substance, dose and duration. To fulfil these requirements pain assessment is of great necessary.

Several studies regarding animal pain assessment have been presented, but until a decade ago only a few of these studies related to pain assessment in horses. Scientists have made progress in this field during the last years and research is still going on. Nevertheless, no universal equine pain scale has yet been developed (Gleerup & Lindegaard, 2015). Horses (and other animals), in contrast to humans, are not able to communicate their pain experience in words to us. Consequently, the pain assessment relies on the observer’s ability to interpret physiological and behaviour indices, thereby collecting indirect evidence of pain (Price et al., 2003). Assessment of pain in horses is complicated further by the circumstance that horses are prey animals and may hide their responses to pain when humans are present.

Recent research has shown that horses change their facial expression during pain, expressing a so called pain face (Gleerup et al., 2015; Dalla Costa et al., 2014). Facial expression of pain has earlier been described and used as a pain assessment tool in other animals such as mice (Langford et al., 2010), rats (Sotocinal et al., 2011) and rabbits (Keating et al., 2012). Using facial expression of pain as a pain assessment tool in horses is not validated but it is of great interest since studies in humans have shown that it is not possible to fully hide facial expression of pain (Prkachin & Mercer, 1989).

The aim of this study is to investigate the clinical application of the equine pain face as a pain assessment tool. The hypothesis was that facial expression of pain in postoperative horses would disappear or diminish after analgesic treatment. The secondary aim of this study is to investigate the reliability and sensitivity of the equine pain face as a pain assessment tool in postoperative horses.

LITERATURE REVIEW

Pain physiology

To be able to collect information about the environment and the organism itself, the body contains highly specialized primary afferents, called sensory fibres (Meyer et al., 2006). Some of the sensory fibres are selectively sensitive to different kind of stimuli (e.g. heat, cold and pressure). Furthermore, a group of sensory fibres have a relatively high threshold, only responding to intense noxious stimuli that could produce injury (intense thermal, chemical and mechanical stimuli), and therefore they are named nociceptors (Meyer et al., 2006). The nociceptors are found in most of the organs in the body and are highly present in the skin,
mucosa, cornea, eardrum and the dental pulp (Norrbrink & Lundeberg, 2014). Internal organs have more diffuse sensory innervations and most of the sensory fibres are found in the fascia surrounding the organs. The pain that originates from the internal organs is called visceral pain (Sjaastad et al., 2010). Furthermore, somatic pain originates from the skin (superficial somatic pain) or from the skeletal muscles, connective tissue, bones and joints (deep somatic pain).

Figure 1. Combined sketch of signal pathway from the periphery all the way to the cortex. Only the pathway via the spinothalmic tract is shown due to simplification. By Bettina Guebeli (Own work) [CC BY-SA 4.0 (http://creativecommons.org/licenses/by-sa/4.0)], via Wikimedia Commons. Available from: https://upload.wikimedia.org/wikipedia/commons/7/70/Sketch_colored_final.png

The nociceptors can be subdivided regarding to the grade of myelinisation, type of activating stimuli, response characteristics and distinct chemical markers (Meyer et al., 2006). Literature often refers to two groups of nociceptors; A-delta-fibre nociceptors and C-fibre nociceptors (Norrbrink & Lundeberg, 2014). C-fibres are unmyelinated and therefore have a slow conduction velocity (< 2m/s), while A-delta-fibres, which are myelinated, have a faster conduction (> 2m/s) (Meyer et al., 2006). Therefore, the pain experience will depend on the fibres that are activated (Norrbrink & Lundeberg, 2014). Activation of A-delta-fibre nociceptors will cause a nerve impulse that conducts fast to the brain, where it will mostly be projected in the somatosensory cortex. This creates an instantaneous pain experience which is sharp, distinct and well localized. In contrast, a nerve impulse created by activation of C-fibre nociceptors will give a slower conduction and it will be projected more diffuse in the brain,
which creates a more widespread pain experience. The different kind of pain experiences can be illustrated by when a person accidently hit its toe on a piece of furniture. First the person will feel a sharp and distinct pain (conducted by A-delta-fibres) and a few seconds later a diffuse and aching pain occurs (conducted by C-fibres) (Norrbrink & Lundeberg, 2014).

Activation on primary afferent nociceptors will result in signal conduction to the dorsal horn of the spinal cord (Todd & Koerber, 2006). In the dorsal horn the first synapse transmission of the signal occurs and glutamate is suggested to be the principal neurotransmitter. Synapses are formed with neurons located in the dorsal horn, including projections cells (with axons that travel to the brain and transport information to several parts in the brain) and interneurons (with axons that remain in the spinal cord). Interneurons can be divided into inhibitory and excitatory interneurons, however still little is known about their organization and function (Todd & Koerber, 2006). Pain is a complex experience, involving several different pathways when passing the pain signal from the spinal cord to the brain (Bushnell & Apkarian, 2006). This results in activation of multiple regions in the forebrain, which creates the pain experience. An overview of the nociceptive pathway is shown in figure 1.

**Pain classification**

There are several ways to classify pain; by time (acute, chronic), underlying cause (nociceptive, inflammatory, neuropathic, psychogenic or idiopathic) (Norrbrink & Lundeberg, 2014) or from a neurobiological perspective (Woolf, 2010). When described from a neurobiological perspective, pain may be divided into three major groups; nociceptive pain, inflammatory pain and pathological pain.

Nociceptive pain serves as an early warnings system for actual or potential injury (Woolf, 2010). When the nociceptors are activated by intense noxious stimuli an action potential in passed to the spinal cord (Norrbrink & Lundeberg, 2014). In the spinal cord, some of the signals are directed by interneurons, further activating sympathetic and motor neurons. This activation of sympathetic neurons results in changes in the blood circulation in the skin, muscles and viscera and also affects the motility of the intestine and the bladder. Activation of motor neurons is a part of a defence mechanism, known as the reflex withdrawal (Norrbrink & Lundeberg, 2014). An example of reflex withdrawal is when a person removes the hand when accidently touching a hot object (Sjaastad et al., 2010). Signals are also passed from the spinal cord to the central nervous system (Norrbrink & Lundeberg, 2014).

The second kind of pain is the inflammatory pain. Cardinal signs of inflammation are heat, swelling, redness, reduced function and pain. Tissue damage or infection causes release of inflammatory mediators, such as prostaglandins, cytokines, growth factors, and bradykinin. These inflammatory mediators can cause spontaneous pain by direct activation of the nociceptors but also act indirectly by stimulating the release of additional algogenic (pain inducing) agents from inflammatory cells (Cunha et al., 2005; Steranka et al., 1988). Steranka et al. (1988) showed that bradykinin acts as a strong algogenic agent on the nociceptors, while Cunha et al. (2005) showed that cytokines play an important role by activating a distinct sequence resulting in the release of prostanoids and sympathomimetic amines. The inflammatory mediators mentioned earlier not only act as algogenic agents but may also cause sensitization of the nociceptors (McMahon et al., 2006; Cunha et al., 2005; Burgess et al.,
which causes primary hyperalgesia. Primary hyperalgesia is characterized by a lowered nociceptor threshold for thermal and mechanical stimuli at the site of the injury (Hardy et al., 1950). Hyperalgesia plays an important role in inflammatory pain, preventing further damage and thereby promotes recovery of the lesion (Woolf, 2010). During the inflammatory state neuropeptides and neurotrophic factors such as substance P and brain-derived neurotrophic factor (BDNF) are released from central terminals of the primary afferents (Woolf, 1983). By acting as co-transmitters they induce long-lasting changes in spinal excitability (Woolf, 1983). Central sensitization will create a pain experience of a bigger area than the actual injury, also called secondary hyperalgesia (Torebjörk et al., 1992).

The third class of pain is the pathological pain. In contrast to nociceptive and inflammatory pain, which work adaptive and protective, pathological pain is suggested to be inadequate and nonprotective (Devor, 2006). Pathological pain resulting from abnormal function of the nervous system can be divided into two subclasses; neuropathic pain and dysfunctional pain (Woolf, 2010). Neuropathic pain is caused by damage or disease in the nervous system. The exact pathophysiological mechanisms causing neuropathic pain are not fully understood (Woolf, 2010). Injury of primary sensory neurons causes electrical hyperexcitability and abnormal impulse generation, referred as ectopic electrogenesis (Wall & Gutnick, 1974). Wall and Gutnick (1974) showed in their study that nerve injury created a steady ongoing stream of nerve impulses and also caused abnormal responsiveness of mechanical stimuli. Studies have shown that blocking of such ectopic discharges from entering the spinal cord results in absence of pain in neuropathic rats (Yoon et al., 1996; Sheen & Chung, 1993). A later study showed that the level of ectopic discharges is well correlated with pain behaviours in a rat neuropathic pain model, which further supports the theory that spontaneous ectopic discharges are an important mechanism related to neuropathic pain (Chul Han et al., 2000). Dysfunctional pain results from neither damage nor inflammation (Woolf, 2010). This type of pain is associated with clinical disorders such as fibromyalgia, irritable bowel syndrome, tension type headache, temporomandibular joint disease, interstitial cystitis etc. The mechanisms behind dysfunctional pain are still not fully understood, but a recent study reinforce earlier evidence that central sensitization and impaired endogenous modulation system are presented in patient with fibromyalgia, chronic daily headache and myofascial pain (Hilgenberg-Sydney et al., 2015).

Pain assessment in horses

Pain assessment in animals (including horses) is a value judgment relying on behavioural and physiological indices to provide indirect evidence of mental state (Molony & Kent, 1997), thereby relaying on the observers ability and experience. Several different factors influence the pain experience and expression, such as species, breed, individual variations, environmental characteristics and drugs (Flecknell, 2000: see Bussières et al., 2008 p. 294). Consequently, assessment of animal pain is highly species specific (Price et al., 2003), requiring research to be executed on the species of interest.

Behavioural responses to pain

Changes I behaviour may be the parameter most often used to assess animal pain (Rutherford, 2002). It has been suggested that there are some general changes in behaviour regardless of
the type of pain, and some more specific behaviour related to specific diseases (Gleerup & Lindegaard, 2015; Ashley et al., 2005). Decreased weight bearing in horses has been recorded in horses with orthopaedic pain (Lindegaard et al., 2010; Bussières et al., 2008), and can be considered as an example of pain-specific behaviour. Moreover, pawing, flank watching and rolling is associated with horses with colic (Sutton et al., 2013; Graubner et al., 2011).

Several different signs of lethargy, assumed to be more general symptoms of pain (Gleerup & Lindegaard, 2015; Ashley et al., 2005), have been recorded in postoperative horses. Horses with somatic postoperative pain have been shown to spend less time eating, exploring and moving, and more time positioned in the back of the stable, head positioned below withers and expressing pre-defined abnormal behaviours (abnormal standing, abnormal locomotion, abnormal oral activity) (Price et al., 2003). Another research supporting this results, suggested that reduced locomotion is a potential indicator of postoperative pain in horses (Pritchett et al., 2003). Horses that underwent exploratory laparotomy, thereby suffering from visceral and somatic pain, showed changes in their posture (including gross pain behaviours, changes in head position and ear position, location in the stable) and socialization (including less interactive behaviour when human approach and less response to grain offered). Pritchett et al. (2003) also showed that postoperative horses spent significantly more time expressing pain behaviour than control horses. Pain behaviours observed in this study were flank gesture, flehmen, kicking and stretching. Increased restlessness, also considered as general pain behaviour (Gleerup & Lindegaard, 2015; Ashley et al., 2005), have been described in postoperative horses (Price et al., 2003).

**Facial expression of pain**

One of the most recent observed behaviour changes in horses during pain is facial expression of pain (a so called pain face). Facial expression of pain is considered to be a useful tool in pain assessment in the non-verbal human population (e.g. cognitive impairment and neonates) (Jordan et al., 2011; Grunau & Craig, 1987). Studies have shown that humans naturally focus on the face when assessing pain in humans and animals (Leach et al., 2011; Williamdes, 2002). Furthermore, studies in humans indicate that facial expression of pain is very hard or impossible to fully hide (Prkachin & Mercer, 1989). All this makes facial expression of pain an interesting approach in pain assessment in horses. Several different studies regarding facial expression of pain related to laboratory animals and rodents have been described. Langford et al. (2010) developed the Mouse Grimace scale, consisting of five so called facial action units (orbital tightening, nose bulge, cheek bulge, ear position and whisker change) (Langford et al., 2010). Later research led to the development of the Rat Grimace Scale (orbital tightening, nose/cheek flattering, ear changes, whisker change) and the Rabbit Grimace Scale (orbital tightening, cheek flattening, nose shape, whisker position, ear position) (Keating et al., 2012; Sotocinal et al., 2011).

<table>
<thead>
<tr>
<th>Table 1. Description of the features of the equine pain face</th>
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<tbody>
<tr>
<td><strong>Pain face feature</strong></td>
</tr>
<tr>
<td>Asymmetrical/ low ears</td>
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of slightly back. The ears may be both asymmetrical and low.

<table>
<thead>
<tr>
<th>Facial Expression</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angled eye</td>
<td>There is tension of the m. levator anguli occuli medialis</td>
</tr>
<tr>
<td>Withdrawn and tense stare</td>
<td>The quality of the glance changes to become withdrawn and tense</td>
</tr>
<tr>
<td>Nostrils – square-like</td>
<td>The nostrils are dilated mediolaterally, especially the medial wing of the nostril may be tense. This is most obvious during inspiration.</td>
</tr>
<tr>
<td>Tension of the muzzle</td>
<td>There is increased tonus of the lips and tension of the chin resulting in an edged shape of the muzzle</td>
</tr>
<tr>
<td>Tension of the mimic muscles</td>
<td>There is tension of the muscles visible on the lateral aspect of the head, especially m. zygomaticus and m. caninus, but m. masseter may also be tense</td>
</tr>
</tbody>
</table>

Facial expression of pain in equines is recently characterized and described almost simultaneously by two different research groups (Dalla Costa et al., 2014; Gleerup et al., 2015). Dalla Costa et al. (2014) noticed six facial action units while recording postoperative horses that had undergone routine castration, resulting in the Horse Grimace Scale (HGS). Their grimace scale involved facial action units; stiffly backwards ears, orbital tightening, tension above the eye area, prominent strained chewing muscles, mouth strained and pronounced chin and strained nostrils and flattening of the profile. Furthermore, the equine pain face is described by Gleerup et al. (2015). They induced noxious somatic stimuli to healthy and un-medicated horses while video recording their faces, and then evaluated alterations in facial expression during the pain experience. All horses that received noxious stimuli showed alterations in facial expressions, even though not all of the identified facial action units were present at all times. Gleerup et al. (2015) summarized the facial action units involved in the equine pain face as “low and/or asymmetrical ears, an angled appearance of the eyes, a withdrawn and/or tense stare, mediolaterally dilated nostrils and tension of the lips, chin and certain mimetic muscles” (table 1 and figure 2). The orbital tightening and backwards ears observed by Dalla Costa et al. (2014) were associated with horses dozing off in the study by Gleerup et al. (2015). Gleerup et al. (2015) therefore suggested that the Horse Grimace Scale describes a combination of pain and fatigue due to the surgical stress response. Moreover, their study showed that horses did not suppress the facial expression of pain when an observer was present but it was less pronounced if the horse tried to interact with the observer.
Physiological responses to pain

Actual or potential threats to an animal (e.g. noxious stimuli) result in well-known stress responses, which allow the animal to allocate bodily resources quickly to resolve a problem (Wiepkema & Koolhaas, 1993). These stress responses include changes in the heart rate, blood pressure, plasma cortisol and endorphin concentrations. Stress responses are affected by many other factors (e.g. fear), and therefore their use as pain indicators is limited when used alone (Conzemius et al., 1997). For example induction of anaesthetic/analgesic treatment alone has been shown to affect the stress response (Benson et al., 2000; Fox et al., 1994). Use of both behavioural and physiological parameters are considered to be more precise than physiological parameters alone (Manteca & Deag, 1993).

Heart rate has long been considered to be a good pain indicator in horses, although researches have presented diverse results. Certain studies have shown good correlation between alteration in heart rate and postoperative pain, acute synovitis and acute somatic pain (Lindegaard et al., 2009; Bussières et al., 2008; Pritchett et al., 2003). However, there are several studies that show no significant difference in heart rate between control group and the group in pain. These studies includes horses with postoperative pain (2011; Sellon et al., 2004; Price et al., 2003; Raekallio et al., 1997) and acute somatic pain (Gleerup et al., 2015). Graubner et al. (2011) stated that “heart rate is sensitive to both internal and environmental factors, such as the temperament of the patients, cardiovascular anomalies, stress, excitement, medication and ileus”. Another physiological parameter investigated is the respiratory rate. Bussieres et al. (2008) managed to show a moderate correlation between respiratory rate and acute synovitis pain, but most of the research done is revealing that respiratory rate in not correlated or poorly correlated with pain (Gleerup et al., 2015; Graubner et al., 2011; Pader et al., 2011; Sellon et al., 2004; Price et al., 2003). Non-invasive systemic arterial blood pressure was suggested to be a good potential pain indicator, due to high specificity and sensitivity in relation to acute synovitis pain (Bussieres et al., 2008). Gleerup et al. (2015)
confirmed this by showing that experimentally induced acute somatic pain raised the mean arterial blood pressure. Investigations into plasma cortisol concentration as a pain indicator have been performed. Like many of the other physiological factors, the outcome of the researches differs. Alteration in plasma cortisol concentration has been described to be a good pain indicator in horses suffering from postoperative pain (Sellon et al., 2004; Pritchett et al., 2003) and acute synovitis pain (Bussières et al., 2008). In contrast, Raekallio et al. (1997) claimed that plasma cortisol is not a good indicator of postoperative orthopaedic pain in horses, since no significant difference was seen between the horses receiving phenylbutazone postoperative and the placebo group.

**Pain scoring scales**

During the last decades several different pain scales have been developed, aimed to assess the severity of the pain of the horse. A pain scale should be estimated to its reliability, sensitivity and validity (Rutherford, 2002). Streiner et al. (2015) described reliability as “an index of the extent to which measurements of individuals obtained under different circumstances yield similar result”. To investigate this the same observer is asked to re-score animals on multiple occasions (intra-observer reliability) or several observers are asked to score the same animals (inter-observer reliability)(Weary et al., 2006). Sensitivity on the other hand is a measure of how the parameter changes with changes in the measured quantity (Natelson et al., 1987). Finally, validity is described as the extent to which a scale actually measures what it is intended to measure (Streiner et al., 2015).

**Unidimensional scales; VAS, NRS and SDS**

The Visual Analogue Scale (VAS) and the Numerical Rating Scale (NRS) are the two most commonly used pain score scales methodologies in humans (Gleerup & Lindegaard, 2015). The VAS consists of a 100 mm line, and is marked “no pain” at one end and “the worst imaginable pain” on the other end, while the NRS is a scale between 0 and 10. The patients decide where to put the marking on the line or scale, to describe the intensity of their pain. When applied to horses an observer is asked to decide the pain intensity, consequently relying on the observer’s ability to interpret pain behaviours of the horse correctly. Therefore, VAS and NRS are well-known to have poor inter-observer reliability (Gleerup & Lindegaard, 2015). The intra-observer reliability is however good, and the VAS and the NRS can be used by the same observer in the same horse over a prolonged time to assess treatment or recovery (Hielm-Bjorkman et al., 2011). The sensitivity of the VAS has been discussed, and the opinions differ. Some researchers claimed that the VAS has higher sensitivity compared to other types of pain scales (e.g. Simple Descriptive Scales) since it is a continuous scale and observers do not have to choose between predefined categories (Reid & Nolan, 1991; Scott & Huskisson, 1976), while others suggested that the continuous scale gives a false impression of having high sensitivity (Holton et al., 1998). Correlation between the VAS and NRS have been suggested to be good (Ahlers et al., 2008), while the agreement between the VAS and a Composite pain Scale (described later) has only been classified as fair (Lindegaard et al., 2010). However, several of the studies mentioned above are not executed on horses, which should be taken in mind when validating the pain scale (Price et al., 2003).
The Simple Descriptive Scale (SDS) consist of description of specific and distinct defined indicators of pain, normally ranked from 0 to 4 or 5 (Gleerup & Lindegaard, 2015), dividing the pain into absent, mild, moderate or severe (Lerche, 2009). Various researchers have been using the SDS when assessing specific types of pain in horses (Fjordbakk & Haga, 2011; Taylor et al., 2002; Jochle et al., 1989). Viñuela-Fernández et al (2011) used a modified version of the Obel scale and a clinical grading system, both simple descriptive scales graded from 0-4 developed to assess equine lameness, to evaluate clinical laminitis in horses. The SDSs used in this study showed overall good reliability. However the inter-observer reliability was lower than the intra-observer reliability (Vinuela-Fernandez et al., 2011). The general problem with the SDS is that all the numerous pain behaviours that exist are not easily fitted into a single five graded scale (Gleerup & Lindegaard, 2015).

**Composite pain scale**

The Composite (Measure) Pain Scale (CPS/CMPS) is a combination of several different SDSs, including specific behavioural or physiological pain indicators, with four or five specific defined grades to each indicator (Gleerup & Lindegaard, 2015). Many of the behavioural and physiological pain indicators described above are included here. Inter-observer reliability and validity have been shown to be high when using a CPS (Sutton et al., 2013; Graubner et al., 2011; Bussières et al., 2008). Bussières et al. (2008) investigated the sensitivity of their CPS and found it possible to distinguish three levels of pain, although they could only classify the sensitivity as good. Not only the pain indicators included separate different CPSs, they also differ in the way of quantifying pain (Gleerup & Lindegaard, 2015). Some researchers have been grading all the pain indicators equally (Sutton et al., 2013; Bussières et al., 2008), while other have weighted different pain indicators according to its significance (Raekallio et al., 1997). The weighted CPM developed by Raekallio et al (1997) has served as inspiration to various other CMPs later developed (Gleerup & Lindegaard, 2015). Gleerup and Lindegaard (2015) recently developed the Equine Pain Scale based on previous pain indicator findings, only including the parameters observed to be the most reliable in the clinic approach. The Equine Pain Scale is not yet validated.

**Grimace scale**

A Grimace scale consists of several facial action units observed when the animal experience pain (Gleerup et al., 2015; Dalla Costa et al., 2014; Sotocinal et al., 2011). The grimace scale has shown good validity when investigated in humans (Langford et al., 2010). Furthermore, studies on laboratory animals and rodents have shown that grimace scales have high reliability and accuracy (Keating et al., 2012; Sotocinal et al., 2011; Langford et al., 2010). The HGS (figure 3) developed by Dalla Costa et al. (2014) showed high inter-observer reliability. Dalla Costa et al. (2014) subjectively graded each facial action unit included in the HGS as; not present (0), moderately present (1) or obvious present (2), and afterwards the total HGS score was calculated. The HGS was well correlated to the CPS also used in the study; a high score of the HGS also resulted in a high score of the CPM (Dalla Costa et al., 2014). However, Gleerup et al. (2015) express the uncertainty whether facial expressions of pain are sensitive for grading pain in a quantitative manner. Both Dalla Costa et al. (2014) and Gleerup et al. (2015) concluded that facial expression of pain could be a good tool for
improving pain assessment in horses, but also suggested further research regarding validity, reliability and sensitivity.

Figure 3. The Horse grimace Scale with images and explanations for each of the 6 facial action units (FAUs). Each FAU is scored according to whether it is not present (score of 0), moderate present (score of 1) and obviously present (score of 2). © 2014 Dalla Costa et al. Originally published in Dalla Costa et al. (2014) under the terms of CC by 4.0 license (http://creativecommons.org/licenses/by/4.0/). Available from: http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0092281.

Management of postoperative pain in horses

Since visceral pain originates from the internal organs (including the gastrointestinal tract) and superficial somatic pain originates from the skin (Sjaastad et al., 2010), postoperative horses that have undergone a ventral midline exploratory laparotomy suffer from both somatic and visceral pain. Horses that have been through abdominal surgery due to acute colic usually receive flunixin meglumine (Sellon et al., 2004), which is a nonsteroidal anti-inflammatory drug (NSAID). Additional medication after surgery is normally just given to horses that express classic behavioural indicators of colic (pawing, flank gestures, rolling etc.). An overview of the analgesics most often used to treat pain in horses is given below.
**Alfa-2 adrenergic drugs**

There are several subclasses of alfa-2 adrenergic agonists. Xylazine and detomidine are the two substances licensed for horses and are commonly used for sedation and analgesia (Robertson & Sanchez, 2010). Xylazine gives excellent visceral analgesia although it has a short duration (up to 90 minutes) (Brunson & Majors, 1987; Muir & Robertson, 1985; Kalpravidh *et al.*, 1984b). Muir and Robertson (1985) induced abdominal pain in nine adult horses by inflating a balloon in the horses’ cecum and evaluated the analgesic effect of xylazine, butorphanol, meperidine and pentazocine. Xylazine was suggested to be the most effective analgesic. Transient hypertension and bradycardia, followed by hypotension, are described adverse effects associated with xylazine (Clark *et al.*, 1988). Detomidine has also been proven to be an effective visceral analgesia (Lowe & Hilfiger, 1986: see Robertson & Sanchez, 2010 p. 609). Lowe and Hilfiger (1986) used the cecal balloon distension model and found that the duration of action of detomidine was 13.5 minutes at 0.005 mg/kg, 45.5 minutes at 0.02 mg/kg and 239 minutes at 0.16 mg/kg. This provides the information that the analgesic duration of detomidine is dose-dependent. Side effects associated with detomidine are ataxia, bradycardia and decreased respiratory rate (Freeman & England, 2000; England *et al.*, 1992).

Generally, alfa-2 adrenergic agonists have been shown to decrease the gastrointestinal motility and to have a relatively short duration of analgesia provided (Elfenbein *et al.*, 2009; Freeman & England, 2001; Doherty *et al.*, 1999; Merritt *et al.*, 1998). This makes the alfa-2 adrenergic agonists less attractive for pain management in postoperative horses. A combination of xylazine and an opioid, such as butorphanol, have been shown to give synergetic analgesic effects (Robertson & Muir, 1983), making it possible to reduce the dose of xylazine. However, the combination of xylazine and butorphanol prolongs the duration of decreased cecal and duodenal activity when compared to only xyalzine given (Merritt *et al.*, 1998; Rutkowski *et al.*, 1991). Finally, the effect of alfa-2 adrenergic agonists administrated as a constant rate infusion (CRI) instead of boluses has not been fully investigated in postoperative horses (Robertson & Sanchez, 2010).

**Nonsteroidal anti-inflammatory drugs**

Nonsteroidal anti-inflammatory drugs (NSAID), acting by inhibit the prostanoid biosynthesis, are commonly used for their analgesic and anti-inflammatory effect. NSAIDs can be divided into traditional NSAIDs (inhibiting both COX-1 and COX-2) and coxibs (selective COX-2 inhibitors) (Bruno *et al.*, 2014). The most commonly used NSAIDs in the veterinary practice are aspirin, ibuprofen, phenylbutazone, flunixin meglumine, ketoprofen, carprofen, etodolac, and meloxicam (Moses & Bertone, 2002). Flunixin meglumine (a traditional NSAID) have been shown to provide effective visceral analgesia (Cook *et al.*, 2009), and is suggested to be one of the most important and commonly used medication for treatment of visceral pain in horses (Robertson & Sanchez, 2010). Flunixin is also proven to reduce lameness and heart rate when administrated as a single dose infusion in horses with foot lameness (Foreman *et al.*, 2010). Meloxicam (a coxib) has been shown to reduce postoperative pain score in horses with experimentally induced ischemic-injured jejunum, when administrated preoperative (Little *et al.*, 2007). Furthermore, meloxicam reduced postoperative somatic pain in horses that underwent orthopaedic surgery (Walliser *et al.*, 2015). However, postoperative horses with small intestine lesions treated with meloxicam (a coxib) have been showed to more often
express gross pain signs, compared to horses treated with flunixin meglumine (Naylor et al., 2014).

Adverse effects in horses associated with NSAID are prevented recovery of ischaemic-injured jejunum (Cook et al., 2009; Tomlinson et al., 2004), prolonged permeability defect in intestinal mucosa (Tomlinson & Blikslager, 2005), renal crest necrosis, decreased total serum protein and albumin concentrations and gastric and colon ulcers (MacAllister et al., 1993). However, there are some studies that indicate that traditional NSAIDs and coxibs differ in the amount of adverse effects. Tomlinson and Blikslager (2005) showed that deracoxib (a selective COX-2 inhibitor) caused no increase in the permeability of the jejunum mucosa in contrast to flunixin meglumine (a traditional NSAID), in horses with experimentally induced ischemic lesion of the jejunum. Furthermore, Cook et al. (2009) showed that the delayed mucosal recovery in experimentally induced ischaemic-injured jejunum, present when using flunixin meglumine, was not detectable when using firocoxib (a specific COX-2 inhibitor), and therefore they suggested that that firocoxib may be useful in horses recovering from ischemic-injured jejunum. However, a study containing horses with naturally occurring strangulation of the small intestine showed no differences in overall survival, postoperative ileus or lipopolysaccharide concentrations between horses treated with meloxicam and flunixin meglumine (Naylor et al., 2014).

**Opioids**

Opioids practice their analgesic effect by acting on specific opioid-receptors throughout the body, brain and spinal cord (Kohn & Muir, 1988). There are several different opioids, acting on different groups of opioid-receptors (named mu, kappa, sigma and delta) (Kohn & Muir, 1988) and with different potency (Kalpravidh et al., 1984b). Most of the opioids used in horses are µ-agonists (morphine, fentanyl, meperidine, oxymorphone and methadone), or mixed agonists/antagonists (butorphanol and pentazocine) (Steven, 1986). Both µ-agonist and κ-agonist have been shown to produce dose-dependent analgesia and increased locomotor activity (Kamerling et al., 1988; Kamerling et al., 1985). However, µ-agonists also caused tachycardia, tachypnea and behavioural arousal (Kamerling et al., 1985) while the κ-agonist showed no changes in respiratory rate and heart rate and horses seemed to experience sedation rather than arousal (Kamerling et al., 1988). This indicates that different opioids have different physiological effects. However, the studies by Kamerling et al. (1985; 1988) are performed on healthy horses with experimentally induced pain; therefore the adverse effect of µ-agonists and κ-agonist in horses with naturally occurring pain is not investigated.

Butorphanol, a κ-agonist and competitive µ-antagonist, provides good visceral analgesia (Kalpravidh et al., 1984b) and have a duration between 15-90 minutes (Kalpravidh et al., 1984a). However, when administrated as an intravenous bolus adverse effects have been observed; such as ataxia, decreased gastrointestinal sounds and decreased defecation (Sellon et al., 2001). When butorphanol was administrated as an intravenous CRI ataxia was not present and the adverse gastrointestinal effects were less apparent compared to the bolus dose. Therefore, Sellon et al. (2001) suggested that butorphanol as a CRI may be a useful treatment of pain in horses. Postoperative CRI of butorphanol have been proven to cause significantly improved behavior scores in horses during the first 24 hours after celiotomy, thereby indicating that the horses experienced less pain (Sellon et al., 2004).
Morphine, an example of a μ-agonist, has been shown to provide analgesia in colic horses (Phaneuf et al., 1972). Kalpravidh et al. (1984b) investigated the analgesic effects of morphine in horses and found that morphine had a good analgesic effect in somatic pain and acceptable analgesia for visceral pain. Morphine is associated with adverse effects such as decreased gastrointestinal sounds, delayed defecation, promoted fecal drying, and CNS excitation (Roberts & Argenzio, 1986). These adverse effects are observed in otherwise healthy horses without pain. When given as a CRI morphine was proved to provide analgesia in horses with carpal synovitis (Carregaro et al., 2014). However, adverse effects such as increased cardiovascular and respiratory parameters and reduced gut sounds were still present.

Sodium channel blockers

Lidocaine is known as local anaesthesia but is commonly administrated as an intravenous CRI in horses for its potential analgesic, prokinetic and anti-inflammatory effects (Robertson & Sanchez, 2010). Human studies have shown that patients that receive lidocaine as an induction bolus and CRI during operation and postoperative, experience less postoperative pain, receive prokinetics effects and need a shorter hospitalization (Kaba et al., 2007; Groudine et al., 1998; Cassuto et al., 1985).

Few studies are done regarding lidocaine as a potential analgesic in horses. Robertson et al. (2005) used a heat element placed on the skin and an intraduodenal and rectal balloon to experimentally induce somatic and visceral pain in healthy horses. Horses that received intravenous lidocaine showed a higher thermal threshold, suggesting that lidocaine play a role in somatic analgesia in horses. However, there was no significant change in tolerance to the experimentally induced visceral pain (Robertson et al., 2005). Robertson et al. (2005) discuss whether the fact that the study only contained horses with normal gastrointestinal function and only one single dose regime could be the reason of the lack of effect. They suggested that inflammatory pain and sensitization are probably present in clinical colic, which could explain the reported efficacy of lidocaine in those patients. Lidocaine is suggested to have anti-inflammatory effects (Nishina et al., 1998; Sasagawa, 1991; Peck et al., 1985), and it is possible that lidocaine exert its positive effects through that. Intravenous lidocaine treatment have been suggested to reduce the occurrence of postoperative ileus (POI) (Torfs et al., 2009; Briaenceau et al., 2002) and have positive effects on already existing POI in horses (Malone et al., 2006). POI can cause severe pain in postoperative horses, and thereby lidocaine can work indirectly as a postoperative analgesic. Adverse effects of lidocaine such as skeletal muscle tremors, altered visual function, anxiety, ataxia, collapse and electrocardiographic changes, are related to intoxication (Meyer et al., 2001).

MATERIALS AND METHODS

Study design

The study was designed as an observational case study. Video recording of the horses was performed at least 24 hours after surgery to minimize the risk of therapy used before and during operation to affect the pain status of the horse on the test day. Each horse was filmed at two occasions, directly before receiving analgesic treatment by intravenous infusion and two
hours after the analgesic infusion, and thereby acting as its own control. The horse was stabled in a box, free to move as it liked, during the whole procedure. The video recordings were blinded and randomized by giving each sequence a random number between 1 and 56. The sample size first chosen was 20 horses, but due to few incoming colic patient during the time data was collected only 12 horses were included.

**Animals**

Twelve horses that underwent ventral midline exploratory laparotomy or routine castration at the university animal hospital in Uppsala (UDS) between May 2015 and October 2015 were included in the study. Inclusion criteria for the study required the patient to undergo surgery which results in visceral and somatic pain and to receive postoperative analgesic treatment. Eleven of the horses included underwent ventral midline exploratory laparotomy due to acute colic, and one horse underwent routine castration. Surgical diagnoses of the horses included are shown in table 2. Four of the horses that underwent exploratory laparotomy had resection of the small intestines and one horse had partial wall resection of the pelvic flexure/right dorsal colon. The horse that underwent routine castration was included due to its postoperative complications such as internal bleedings and severe pain, which is comparable with the pain after exploratory laparotomy.

All horses that underwent exploratory laparotomy received preoperative treatment with bensylpenicillin (18-20 mg/kg), gentamicin (6.5-6.6 mg/kg) and flunixin (0.8-1.1 mg/kg), except one horse that only received bensylpenicillin and gentamicin. Further, they received romifidine (0.05-0.1 mg/kg) and butorphanol 0.02-0.03 mg/kg) as premedication, except one horse that just received romifidine. Three of the horses that underwent exploratory laparotomy also received acepromazine as premedication (0.02-0.03 mg/kg). The induction was carried out by diazepam (0.03-0.04 mg/kg) and ketamine (2.1-2.2 mg/kg) and the anaesthesia was maintained with isoflurane. Two horses needed additional medication during maintenance and received xylazine (0.1 mg/kg). Futhermore, two of the horses that underwent exploratory laparotomy received lidocaine as a bolus dose (2 mg/kg) followed by constant rate infusion (2 mg/kg/h) during anaesthesia. One of the horses also received pentobarbital (0.009 ml/kg) and romifidin (0.03 mg/kg). The record of the anaesthesia was lost of three of the horses that underwent exploratory laparotomy. The horse that underwent routine castration received preoperative bensylpenicillin (19 mg/kg) and flunixin (1.1 mg/kg) followed by acepromazine (0.03 mg/kg), romifidine (0.1 mg/kg) and butorphanol (0.02 mg/kg) as premedication. Further, induction was carried out by diazepam (0.03 mg/kg) and ketamine (2.2 mg/kg) and the anaesthesia was maintained by isoflurane. Detailed information about the horses included, such as age, sex and breed are summarized in table 2. Informed owner consent was obtained for each horse.

Table 2. Details of the horses included in the study

<table>
<thead>
<tr>
<th>Horse</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Weight (kg)</th>
<th>Breed</th>
<th>Surgical diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>Gelding</td>
<td>405</td>
<td>Dutch import</td>
<td>Adhesion formation between the cecum, abdominal wall and pelvic flexure/ left dorsal colon</td>
</tr>
</tbody>
</table>
Data collection

The medical records of the horses included in the study, containing information about signalement (age, breed, sex), history of the current episode of colic, physical examination during hospitalization, laboratory results, clinical diagnosis and treatment of the patient (performed surgery, date of the surgery, surgical diagnosis including disease location), and medical therapy (anaesthesia, analgesia and postoperative treatment), were compiled into an Excel file and analysed using Excel and Minitab. The analgesic treatment at the time of film recording for each horse is summarised in table 3.

Table 3. Analgesic treatment of the included horses at the time of film recording

<table>
<thead>
<tr>
<th>Horse</th>
<th>Film occasion</th>
<th>Analgesic treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>Lidocaine CRI 3 mg/kg/day</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Lidocaine CRI 3 mg/kg/h, flunixin 1.1 mg/kg 1 time/day</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Flunixin 1.1 mg/kg 1 time/day</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>None</td>
</tr>
</tbody>
</table>
2
Flunixin 1.1 mg/kg 1 time/day

4 1
Lidocaine CRI 3 mg/kg/h
2
Lidocaine CRI 3 mg/kg/h, flunixin 1 mg/kg 1 time/day

5 1
None
2
Flunixin 1.1 mg/kg 1 time/day

6 1
None
2
Flunixin 1.1 mg/kg 2 times/day

7 1
Flunixin 1.1 mg/kg 1 times/day
2
Detomidine 0.007 mg/kg, butorphanol bolus 0.02 mg/kg + butorphanol CRI 12.5 mg/kg/h

8 1
Lidocaine CRI 3 mg/kg/h
2
Lidocaine CRI 3 mg/kg/h, flunixin 1.1 mg/kg 2 times/day

9 1
Lidocaine CRI 3 mg/kg/h
2
Lidocaine CRI 3 mg/kg/h, flunixin 1 mg/kg 2 times/day

10 1
Lidocaine CRI 3 mg/kg/h
2
Lidocaine CRI 3 mg/kg/h, flunixin 1.2 mg/kg 2 times/day

11 1
Butorphanol CRI 0.025 mg/kg/h
2
Butorphanol CRI 0.025 mg/kg/h, flunixin 1 mg/kg 1 time/day

12 1
Lidocaine CRI 3 mg/kg/h
2
Lidocaine CRI 3 mg/kg/h, flunixin 1.1 mg/kg 2 times/day

**Video recordings**

The video recording was performed by a single person (the author) using a pocket type digital camera and a tripod. Disturbance of the horse during the procedure attempted to be avoided by working quietly and when possibly stand out of the view of the horse. The tripod with its camera was placed outside the box, approximately 1.5-2 meters from the box. The horse and its behaviour were filmed from this view for 2 minutes. After two minutes the person walked towards the door of the box, opened it and offered the horse a small piece of an apple by reaching out the hand and not making eye contact, and thereby recording the interactive behaviour and response to food. The whole procedure was filmed by the camera on the tripod. The camera was then removed from the tripod to enable a close up filming of the face of the horse. The halter was removed from the horse before filming the face. The camera was held so that the camera caught the whole face of the horse for at least two minutes if possible. A label with the name and journal number of the horse and a watch were filmed to be able to connect the film with the correct medical journal, time and date. This whole procedure was repeated when filming the horse two hours after analgesia administration.
Video editing

Both films of each horse, one before treatment and one after treatment, were cut down to three different film sequences by using Camtasia studio 8® software. The first sequence contained a 90 seconds long film involving the horse viewed from outside the box, evaluation of its interactive behaviour and its response to food. The film sequence selection and editing was made so that the sequence represented the behaviour of the horse of the full time film. Consequently, if the horse showed signs of pain only half of the time filming, both normal behaviour and pain behaviour must be included in the final sequence. The second sequence contained a 2 minutes long close up film of the face of the horse. The third sequence contained 30 seconds close up film of the face of the horse, the best part of sequence number two. The film clips of the face included were subjectively chosen by a single non-blinded person. This non-blinded person subjectively selected a sequence that involved as many facial action units of the equine pain face as possible. To be selected the film sequence also had to be of good quality. In a few cases it was not possible to film the face of the horse for 2 minutes (horse refused to stand still), and the recorded film therefore were put on repeat to receive the 2 minute sequence.

All the film sequences were first put into two different PowerPoint presentations, the 90 seconds clips in the first PowerPoint presentation and the 2 minutes clips and 30 seconds clips of the horses’ faces in the second PowerPoint presentation. Each film clip was inserted at a separate slide in the PowerPoint presentation, in a randomized order. The randomisation was carried out by using a list randomizer at a website. Eight of the twelve horses included in the study were selected randomly by using the randomizer list. To be able to evaluate the intra-observer reliability the 2 minute clip before analgesic treatment of each selected horse were chosen to be inserted two times in the second PowerPoint presentation.

A third PowerPoint presentation was made, containing only the 30 seconds clips of the horses’ faces. Each horse, before and after treatment, was inserted on a separate slide in a randomised order inside the slide, and labelled with the letters A and B.

Observer blinded pain scoring

Table 4. Modified version of the Equine pain scale

<table>
<thead>
<tr>
<th>Behaviour category</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Gross pain behaviour*</td>
<td>None</td>
</tr>
<tr>
<td>Activity</td>
<td>Exploring attention towards the surroundings or resting</td>
</tr>
<tr>
<td>Location in the stall</td>
<td>At the door watching the environment</td>
</tr>
</tbody>
</table>
### Posture/weight bearing

| Normal posture and normal weight bearing | Foot intermittent of the ground/occasional weight shift | Continuously taking foot off the ground and trying to replace it. | No weight bearing. Abnormal weight distribution |

### Head position

| Foraging, below withers or high | Level of withers | Below withers |

### Attention towards the painful area

| Does not pay attention to painful area | Brief attention to painful area (e.g. flank watching) | Biting, nudging or looking at painful area (e.g. flank watching) |

### Interactive behaviour

| Looks at observer or moves to observer when approached | Looks at observer does not move | Does not look at observer or moves away avoids contact | Does not move, not reacting/ introverted |

### Response to food

| Takes food with no hesitation | Looks at food | No response to food |

*Gross pain behaviour includes all readily visible behaviours like, excessive head movements (vertical/lateral), flehmen, kicking, pawing, rolling, tail swishing, mouth playing, repeated stretching, etc. © 2015 EVJ Ltd. Adapted with permission from Gleerup, K.B. & Lindegaard, C. (2015), Recognition and quantification of pain in horses: A tutorial review. Equine Veterinary Education. doi: 10.1111/eve.12383. Published online by Wiley Online Library.

The two first PowerPoint presentations were shown to two different groups of blinded observers; a group of veterinarians consisting of three individuals and a group of veterinary students consisting of four individuals. The three veterinarians had a large experience of pain assessment in horses by evaluating facial expression. The students, which did not have any experience of pain assessment in horses by facial expression, received a 30 minutes long introduction, given by the author, about facial expression of pain in horses just before they watched the movies of the faces of the horses. The PowerPoint presentation containing the 90 seconds clips were shown the first day. The observers were asked to pain score the horses included by using the modified version of the Equine Pain Scale (pain face category and “pinched” alternative excluded, hereafter called the modified EPS) (table 4). Each clip was shown only ones. If the observers were not able to assess a certain category (e.g. due to obscuring view) they were asked to draw a line in the box.

At the second day the PowerPoint containing the 2 minutes clips and 30 seconds clips were shown to the same observers, who were asked to grade the facial expression of each horse in “no pain face”, “pain face present” or “intense pain face”. Each film sequence was shown only ones and the observers had to choose an answer to each horse. The veterinarians were also asked to motivate what they were basing their quantitative assessment of the pain face on. The veterinary students, after receiving their introduction about facial expressions of pain
in horses, were asked to grade the facial expression of pain in the categories mention above by using Turning point ResponsCards.

The third PowerPoint presentation was shown to a new group of observers (6 veterinarians). They were asked to tell which of the horses on the same slide that was in most pain, or if the horses were in same grade of pain. Observers were allowed to play the film sequences as many times they wanted.

**Statistical analysis**

Minitab 17 © statistical software was used to perform the statistical analysis. Fleiss’ kappa was used to calculate inter- and intra-observer reliability of the grading of facial expression of pain. Interpretation of the kappa values was based on a table suggested by Landis and Koch (1997). Since both experienced and inexperienced observers were shown the film sequences the differences in inter- and intra-observer reliability was evaluated.

Only observers with an intra-observer reliability of facial expression of pain with the kappa value of 0.5 or more were selected (five observers) to represent further investigations, since they were appraised to have a more reliable way of grading facial expression of pain. The sensitivity of facial expression of pain was investigated by comparing the medians of facial expression of pain with the median scores of the modified EPS, and also by evaluating the inter-observer reliability of each grading category of facial expression of pain. Correlation between grading of facial expression of pain and the score of the modified EPS was calculated, using Kendall’s tau-b.

To investigate the hypothesis of this study the median score of facial expresses of pain and the median score of the modified EPS were compared within each horse before and after analgesic treatment. A paired t sample test was performed of the modified EPS score before and after treatment. Furthermore, Fisher’s exact test was calculated for grading of facial expression of pain (no pain face, pain face present) before and after treatment.

To investigate if it is possible to see facial expressions of pain after watching the face for just 30 seconds, Fleiss kappa was used to compare the agreement between the 30 seconds clips ad 2 minutes clips. The effect of the lidocaine and butorphanol hangover treatment was investigated regarding the proportion of present pain face and agreement between observers. Only the results of observers with a kappa value of intra-observer reliability above 0.5 was included. Agreement was calculated by counting the proportion of observers agreeing with the median value of the grading of facial expression of pain in each horse. Finally, inter-observer reliability was also calculated of the observers who graded which horse that was in most pain (the third PowerPoint presentation).
RESULTS

Facial expression of pain

Inter-observer reliability

The kappa value of the overall inter-observer reliability of grading facial expression of pain for all observers was determined to 0.11 (slight agreement) when three categories were used to grade the facial expression of pain (no pain face, pain face present, intense pain face). An increase of the kappa value to 0.22 (fair agreement) was seen when only two categories were used in the grading (no pain face, pain face present). Both inexperienced and experienced observers increased their inter-observer reliability when only two categories were used to grade facial expression of pain. Observers agreed in a higher amount of the horses that showed no pain face or intense pain face, compared to when horses just expressed a present pain face. The kappa values of each category are shown in table 5. Experienced observers had a higher agreement of the category intense pain face (kappa value 0.22) compared to inexperienced observers (kappa value 0.04) and experienced observers stated to base their quantitative assessment on the number of visible facial action units and the overall impression.

<table>
<thead>
<tr>
<th>Grading</th>
<th>Kappa</th>
<th>SE Kappa</th>
<th>Grading</th>
<th>Kappa</th>
<th>SE Kappa</th>
</tr>
</thead>
<tbody>
<tr>
<td>No pain face</td>
<td>0.218973</td>
<td>0.0291606</td>
<td>No pain face</td>
<td>0.218973</td>
<td>0.0291606</td>
</tr>
<tr>
<td>Pain face present</td>
<td>0.025588</td>
<td>0.0291606</td>
<td>Pain face present</td>
<td>0.218973</td>
<td>0.0291606</td>
</tr>
<tr>
<td>Intense pain face</td>
<td>0.105886</td>
<td>0.0291606</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td>0.112651</td>
<td>0.0216156</td>
<td>Overall</td>
<td>0.218973</td>
<td>0.0291606</td>
</tr>
</tbody>
</table>

The inter-observer reliability was also calculated of the observers grading which horse that was in most pain (third PowerPoint presentation). The overall kappa value was determined to 0.48 (moderate agreement) (see table 5).

<table>
<thead>
<tr>
<th>Which horse is in most pain?</th>
<th>Kappa</th>
<th>SE Kappa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Horse before treatment</td>
<td>0.541259</td>
<td>0.0745356</td>
</tr>
<tr>
<td>Horse after treatment</td>
<td>0.444444</td>
<td>0.0745356</td>
</tr>
<tr>
<td>Both are in the same pain</td>
<td>0.373913</td>
<td>0.0745356</td>
</tr>
<tr>
<td>Overall</td>
<td>0.483871</td>
<td>0.0667585</td>
</tr>
</tbody>
</table>

Intra-observer reliability

The mean kappa value of all observers was determined to 0.51 (moderate agreement). The kappa values of the inexperienced observers varied and were determined to -0.33 (poor agreement), 0.53 (moderate agreement), 0.71 (substantial agreement) and 0.77 (substantial agreement). The mean kappa value of the inexperienced observers was 0.42 (moderate agreement). The experienced observers had the following kappa values; 0.37 (fair agreement),
0.51 (moderate agreement) and 1 (perfect agreement). This gave a mean kappa value of 0.63 (substantial agreement). The intra-observer reliability is summarized in table 7.

Table 7. Kappa values of intra-observer reliability

<table>
<thead>
<tr>
<th></th>
<th>Inexperienced</th>
<th>Experienced</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kappa</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-0.333333</td>
<td>Poor agreement</td>
<td>0.372549</td>
</tr>
<tr>
<td>0.52941</td>
<td>Moderate agreement</td>
<td>0.507692</td>
</tr>
<tr>
<td>0.709091</td>
<td>Substantial agreement</td>
<td>1</td>
</tr>
<tr>
<td>0.76812</td>
<td>Substantial agreement</td>
<td></td>
</tr>
<tr>
<td>Mean kappa 0.42</td>
<td></td>
<td>Mean kappa 0.63</td>
</tr>
</tbody>
</table>

**Agreement between the 30 seconds clips and the 2 minutes clips**

The median of facial expression of pain of the 30 seconds clips and 2 minutes clips were used to calculate Fleiss’ kappa. Agreement between the 30 seconds clips and the 2 minutes clips was determined to 71%, with an overall kappa value of 0.44.

**Effect of analgesic treatment**

The analgesic treatment by time of the film occasions differs between the horses (see table 3). To investigate how the lidocaine and butorphanol hangover treatment affect the grading of facial expression of pain the proportion of expressed facial expression of pain and agreement between observers in the different treatment groups was calculated (see table 8).

Table 8. The effect of lidocaine and butorphanol treatment on proportion of present pain face and agreement between observers. Based on the assessment by observers with an kappa value of intra-observer reliability above 0.5

<table>
<thead>
<tr>
<th>Horse</th>
<th>Film occasion</th>
<th>Grading of pain face</th>
<th>Agreement</th>
</tr>
</thead>
<tbody>
<tr>
<td>No lidocaine/butorphanol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>Present</td>
<td>80%</td>
</tr>
<tr>
<td>2</td>
<td>No</td>
<td></td>
<td>100%</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>Present</td>
<td>80%</td>
</tr>
<tr>
<td>2</td>
<td>Present</td>
<td>60%</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>Present</td>
<td>80%</td>
</tr>
<tr>
<td>2</td>
<td>No</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>No</td>
<td>60%</td>
</tr>
<tr>
<td>2</td>
<td>Present</td>
<td>60%</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>Present</td>
<td>100%</td>
</tr>
</tbody>
</table>

Proportion pain face: 67%   Mean agreement: 80%
To decide whether the horses expressed facial expression of pain the median of grading of facial expression of pain, by the observers with a kappa value of intra-observer reliability above 0.5, was calculated for each horse before and after treatment. Nine out of twelve horses included in the study expressed facial expression of pain before analgesic treatment. Of these horses, four did not express facial expression of pain after analgesic treatment and five horses did not show any changes in grading of facial expression of pain. Three of the horses included in the study showed no facial expression of pain before analgesic treatment but in contrast the expressed it after analgesic treatment. The distribution of the horses is shown in table 9. No significant difference could be seen in presence of facial expression of pain before and after analgesic treatment when Fisher’s exact test was calculated (p-value = 0.68).

Table 9. Cross-tabulation of grading of facial expression of pain before and after analgesic treatment

<table>
<thead>
<tr>
<th>Before treatment</th>
<th>After treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No pain face</td>
</tr>
<tr>
<td>No pain face</td>
<td>0</td>
</tr>
<tr>
<td>Pain face present</td>
<td>3</td>
</tr>
<tr>
<td>Intense pain face</td>
<td>1</td>
</tr>
</tbody>
</table>
The equine pain scale

Effect of analgesic treatment

The pain score assessment of each horse before and after analgesic treatment, made by observers with a kappa value above 0.5, is presented in figure 3. The median values of the score of the modified EPS were calculated for each horse (before and after analgesic treatment), to be able to compare the score before and after treatment. Analgesic treatment caused a decrease in pain score in 50% of the horses, an increase in pain score in 33% of the horses and no difference in pain score in 17% of the horses. However, no significant difference could be seen in pain score before and after analgesic treatment.

Relationship between facial expression of pain and the Equine Pain Scale

The total pain score of the Equine Pain Scale was divided into three different groups by using the boxplot of pain score. Group 1 consisted of median pain score of 0-3, group 2 consisted of median pain score of 4-7, and group 3 consisted of median pain score of 8-11. These groups of horses were compared to the grading of facial expression of pain. 63% of the horses in group 1 expressed a pain face and 60% of the horses in group 2 expressed a pain face. Furthermore, 100% of the horses in group 3 expressed a pain face. Kendall’s Tau-b was determined to 0.3 when facial expression of pain (no pain face, pain face present and intense pain face) was compared to the Equine Pain Scale score (group 1, 2 and 3).

Figure 3. Boxplot of the Equine Pain Scale score before and after treatment, decided by the observers with an intra-observer reliability kappa value over 0.5.

[Boxplot image]
DISCUSSION

Study design

This study evaluates the clinical application and usefulness of facial expression of pain as a pain assessment tool in postoperative horses. Facial expression of pain has earlier been described in horses with experimentally induced somatic pain and in horses after routine castration (Gleerup et al., 2015; Dalla Costa et al., 2014), but the facial action units described differs slightly between the studies. For this study the equine pain face described by Gleerup et al. (2015) was assumed to be the most reliable, since the HGS described by Dalla Costa et al (2014) is suggested to be a combination of fatigue and pain (Gleerup et al., 2015). Reference population of this study unit aims to be postoperative hospitalised horses with combined visceral and somatic pain.

Previous studies of facial expression of pain in horses have been performed during more controlled and experimental conditions. Dalla costa et al (2014) used two high definition cameras placed at opposite sides of the box, which enabled capturing of high quality videos and still images. Furthermore, the horses in the study by Gleerup et al (2015) were filmed while they were fixated by a neck collar and with a bright background of the face to make the facial expression more visible. In the present study, performed to resemble the clinical practice, horses were not fixated during filming of the face and the light was not always optimal to capturing the subtle changes in facial expression. Other factors that may have contributed to a more difficult assessment of facial expression of pain compared to previous studies are the fact that 3 of the horses included received skin lesion and swellings around the eyes during anaesthesia induction, one of the horses received postoperative toxinemia and secondary oedema of the face and one horse had underwent a pinna resection of the left ear. Some specific horses in the present study (horse five and six) were classified by the experienced observers as hard to evaluate or to have a sedated look. Horse five continuously moved its head during the film recording and horse six was one of the horses with skin lesions and swellings around the eyes. This shows the difficulties and variation in the ability to correctly assess facial expression of pain in the clinical practice.

A newly publish study has proven that isoflurane anaesthesia alone increase the score of the Mice Grimace Scale (Miller et al., 2015). However, in the study pain assessment was performed only 30 minutes after anaesthesia. All horses included in the present study were filmed at least 24 hours after surgery. Therefore the medical therapy used during anaesthesia should not be able to interfere with the pain assessment.

A modified version of the EPS was used to pain score all the included horses. The observer should pain score the horse in a quiet environment, without interacting or disturbing the horse, to make sure that the horse does not hide its pain behaviours. In an equine practice this in not always possible. There are a lot of persons working at the clinic and there are often several different horses in each stall, consequently there will be interaction and noises around the horses. Furthermore, the observer needs to stand relatively close to the box to be able to assess the pain behaviours of the horse. Therefore, it is possible that the horses in the present study hid some of their pain behaviours and that the pain score is lower than it would be in an optimum environment. However, the environment in the study is realistic and therefore the clinical application of the modified EPS is evaluated. The category “posture/ weight bearing” was hard to evaluate in several video films due to the horse was standing behind the box wall,
making it harder to notice weight shifting. This indicates that the category “posture/weight bearing” sometimes is hard to evaluate in the clinical practice, and that horses may get a lower score of the modified EPS than they would with experimental conditions.

Effect of analgesic treatment

All of the 12 horses included in the present study showed facial expression of pain, either before or after analgesic treatment, or by both film occasions. The hypothesis was that facial expression of pain in postoperative horses would disappear or diminish after pain medication. However, there was no significant difference of either facial expression of pain or score of the modified EPS before and after treatment. Only 4 of the 9 horses that expressed facial expression of pain before treatment responded to the analgesic treatment by not expressing facial expression of pain. Consequently, 5 of the horses continued to exhibit facial expression of pain after analgesic treatment. The reason could be that the analgesic treatment given was not enough to give full-scale analgesia, and therefore the horses were in pain in both film occasions. In the present study the number of expressed facial action units of the equine pain face was not evaluated. Therefore, the possibility that these five horses expressed fewer facial action units after the analgesic treatment cannot be rejected. Another possible reason for horses expressing facial expression of pain after analgesic treatment could be that other conditions than pain can cause changes in the facial mimic of the horse. It is common knowledge that human beings express not only facial expression of pain but also change their facial mimic when they experience nausea, distress, excitement, fear etc. However, these conditions have not yet been investigated regarding facial expressions in horses. Therefore, the specificity of facial expression of pain in horses can be questioned.

The fact that six of the horses included were put on a CRI of lidocaine and one horse received CRI of butorphanol, during both film occasions should be taken into account. Lidocaine has been proven to reduce somatic pain and inflammation (Nishina et al., 1998; Sasagawa, 1991; Peck et al., 1985) and butorphanol provides good visceral analgesia (Kalpravidh et al., 1984b). Consequently, the hangover treatment with lidocaine and butorphanol could have contributed to a lower grade of pain in the horses, both before and after the analgesic treatment, further contributing to the non-significant difference before and after treatment in grading of facial expression of pain and score of the modified EPS. However, horses that did not receive CRI of lidocaine or butorphanol did not show a significant difference before and after treatment either.

Reliability

The inter-observer reliability of grading of facial expression of pain has previously been described as high when used as a grimace scale, with an overall Intraclass Correlation Coefficient value of 0.92 (Dalla Costa et al., 2014). In this present study Fleiss kappa was used to calculate the reliability, therefore the numbers between the studies should not be compared directly. However, in the present study there was only slight agreement between the observers, with a kappa value of 0.11 when the grading consisted of three categories and 0.22 when only two categories are used. The low agreement between observers is suggested to depend on several things, such as differences in the subjective grading of a so called pain face between observers, the overhanging treatment with lidocaine and butorphanol and the
differences between individuals’ ability of grading facial expression of pain. In this present study the observers were asked to grade the facial expression of pain subjectively, without a definition of the two “pain face present” or “intense pain face”. This is most likely the reason for the low inter-observer reliability. By using a grading system for each facial action unit, as in the study by Dalla Costa et al (2014), the assessment gets more objective.

Low doses of lidocaine are described as sedating in some anesthesiology handbooks (Doherty & Valverde, 2006), and κ-agonists cause sedation in healthy horses (Kamerling et al., 1988). Therefore, it is possible that the lidocaine and butorphanol treatment could have caused changes in the facial mimic, making it harder to evaluate facial expression of pain. Treatment with lidocaine or butorphanol is suggested to contribute to a lower inter-observer reliability, since the agreement of grading of facial expression of pain between observers was lower in the groups of horses treated with lidocaine or butorphanol (see table 8).

Furthermore, horses included in the present study were suffering from visceral and somatic pain. The equine pain face was described in horses with experimentally induced somatic pain (Gleerup et al., 2015). It is possible that horses experiencing visceral pain express a different kind of pain face, which could be an additional reason for the low inter-observer reliability.

Inter-observer reliability was also calculated of observers choosing which horse that was in most pain (horse before treatment, horse after treatment or same level of both). Following the pain level of a horse in analgesic treatment is an important and ordinary task in the clinical practice. An increase of the kappa value to 0.48 was seen, indicating that facial expression of pain is easier to grade when compared when compared within a horse by different occasions.

The intra-observer reliability varied between the observers, suggesting that there are individual variations in the ability to evaluate facial expressions of pain. However, 5 out of 7 observers had a kappa value above 0.5 which is considered as moderate agreement. The differences in intra-observer reliability between inexperienced and experiences observers suggest that experienced individuals have a better capacity of grading facial expression of pain. Still, the relatively high kappa values of the inexperienced observers suggest that it is possible to learn the basics of assessment of facial expression of pain after only a short introduction. Statistical analysis of the reliability of facial expression of pain in horses suggests that it could be a valuable assessment tool when the same person follows the pain status of the same patient over a time, for example if a treating veterinarian wants to follow the recovery of a horse.

### Sensitivity

The ability to grade pain in a quantitative manner by using facial expression of pain have been questioned (Gleerup et al., 2015). In this study the observers were asked to subjectively grade the facial expression of pain in “no pain face”, “pain face present” or “intense pain face”. The kappa value of the alternative “pain face present” was determined to 0.03 and the kappa value of the alternative “intense pain face” was determined to 0.11 when investigating the inter-observer reliability. These results suggest that facial expression of pain is a more reliable tool when the horse has an intense pain face, but it is easy to miss a mild or moderate pain face. Dalla Costa et al (2014) showed that horses with high pain score of a Composite Pain Scale
also received high HGS score, suggesting that the number of facial action units of the equine pain face represent the amount of pain. However, no study has been done regarding the signification of each facial action unit of the equine pain face. A weighting of the facial action units would be of great interest to be able to correctly quantify the pain by facial expression.

A comparison was made between the Equine Pain Scale score and grading of facial expression of pain. Facial expression of pain was shown by horses with very different pain score, including horses with pain score of 0. Several different factors influence the pain experience and expression, such as breed, individual variation, environment characteristics and drugs (Flecknell, 2000: see Bussières et al., 2008). Furthermore, horses are prey animals and may hide some of their pain behaviours. However in humans, facial expression of pain has been proven impossible to fully hide (Prkachin & Mercer, 1989). In theory this makes facial expression of pain a more reliable pain assessment tool in the clinical practice, compared to other pain behaviour based scales.

In this present study, five horses with a low pain score of 0-3 expressed a so called pain face. This could indicate that facial expression of pain has a higher sensitivity than the Equine Pain Scale when used in the clinical practice. Still, four horses with a pain score of 6-7 did not express facial expression of pain. These four horses were all difficult to judge due to heavy head movement, bad quality of video recording, swollen eyes of the horse and horse temperament. However, it is impossible to say whether these horses actually experienced pain or not. Furthermore, the Equine Pain Scale is not validated and the sensitivity of the scale is not determined. Therefore, the sensitivity of facial expression of pain in hard to evaluate in this present study. All horses with a pain score of 8 and above expressed facial expression of pain. This could indicate that facial expression of pain is more reliable in horses with a higher grade of pain, or that those horses below pain score of 8 are not always in pain.

**CONCLUSION**

Facial expression of pain was observed in all of the 12 horses included in the present study. However, no significant difference in grading of facial expression of pain or pain score before and after analgesic treatment could be seen. This indicates that the analgesic treatment was not always optimized or that there are several other conditions present that caused changes in the face mimic in horses. Evaluation of the reliability of the grading of facial expression of pain suggests that evaluation of pain face could be a good supplementary pain assessment tool when a single person follows a patient over a time. A definition of the equine pain face regarding the amount of present facial action units needed and weighting of the facial action units is of great interest in order to create a higher inter-observer reliability. A comparison between the grading of facial expression of pain and the Equine Pain Scale score showed that all horses with a pain score of 8 and above showed facial expression of pain. Further studies regarding the specificity and sensitivity of facial expressions of pain are required.
REFERENCES


