Objective pain scales in horses with facial pain

Anne-Marie Sas (BSc)
Supervisors: Dr. Thijs van Loon, Dr. Machteld van Dierendonck
June 2014

Summary

Animal pain is an aversive sensory and emotional experience representing awareness by the animal of damage or threat to the integrity of its tissues; it changes the animal's physiology and behavior to reduce or avoid damage, to reduce the likelihood of recurrence and to promote recovery. To provide every animal with individual tailored pain management, the recognition of pain is very important. Furthermore, the assessment of pain in animals is also very important for clinical evaluation and decision making. Unfortunately pain in animals is very difficult and hard to measure, unlike in verbal humans.

The aim of this study was the construction and internal validation of objective pain scales and comparison of different pain scales in horses with facial pain.

Eight horses of various breeds (3-19 years old) with different painful conditions of the head admitted to the Equine Health Department of the Faculty of Veterinary Medicine in Utrecht between 19/08/2013 and 6/10/2013 were used for this study. Painful conditions included for example uveitis, sinusitis, jaw fractures and post-surgical trauma. The control group consisted of eight horses owned by the Department of Equine Sciences, seven mares and one gelding (4-15 years old, various breeds) which in daily life are used for education.

Three pain scales were used to score the amount of pain: the Composite Pain Scale (CPS), the Facial Expression Pain Scale (FEPS) and the existing Visual Analogue Scale (VAS). Scoring of CPS and FEPS were performed independently at the same time by two observers, not blinded for the reason of admittance or treatment of the case. The VAS score was given by the treating vet at the Equine Clinic. Scoring of the CPS and FEPS was performed at several moments during the days following admittance to monitor the pain and notice the changes in pain in time. Horses that did not undergo surgery where scored at T=0, T=1st morning and T=2nd morning. The cases which underwent surgery were scored at T=0 pre- surgery, T=0 post-surgery, T= 1st morning, T=1st afternoon, T=2nd morning and T=2nd afternoon post-surgery. During pain scoring all horses (cases and controls) were placed in the same standard stable. The FEPS was also scored during one minute in the stable where the cases resided during hospitalization (own stable: FEPSos).

The correlation coefficient between the two observers was highly significant ($p<0.01$) for the CPS (r=0.921**), FEPS (r=0.823**) and FEPSos (r=0.911**). Distribution of FEPSos was not the same between the subgroups and a significant difference in the pain scores of controls and cases existed, which was not seen in the FEPS and CPS score. The FEPSos showed a very high sensitivity (99.99%) and specificity (99.99%) for facial pain. There is no significant correlation between the VAS score (golden standard) and the tested pain scales. In the timelines of all scoring systems (CPS, FEPS, FEPSos) most cases show an increase in pain score at 4/5 hours or 24 hours post-surgery, the pain score gradually drops after 36 hours post-surgery, with exception of some cases.

The conclusion of this study is that a FEPS score in the horse' own stable is the most reliable and useful pain scoring system to assess pain in horses with facial pain.

Contents

Introduction

Pain

According to the International Association for the Study of Pain (IASP) pain is defined as 'an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage'(1). A more specific definition for pain in animals is: 'Animal pain is an aversive sensory and emotional experience representing an awareness by the animal of damage or threat to the integrity of its tissues; it changes the animal's physiology and behavior to reduce or avoid damage, to reduce the likelihood of recurrence and to promote recovery'(15).

There are different types of pain. Nociceptive pain is caused by tissue damage. The nociceptoren localized in the entire body percept pain by direct observation and indirectly by chemical stimulation due to the release of for example prostaglandins (inflammation). This kind of pain can be treated well with NSAIDs (non-steroidal anti-inflammatory drugs) and/or opioids. Somatic pain is nociceptive pain, starting from skin, connective tissue, muscle tissue or bones. It is generally characterized by a localized, sharp, stabbing or throbbing pain. Visceral pain is also nociceptive, originating from intestines in the chest or abdomen. This pain is mostly not clearly localized and gives a piercing, cramping or pressing sensation. Neuropathic pain is pain caused by a lesion or disease of the central or peripheral somatosensory nervous system. It is a clinical description (and not a diagnosis), which requires a demonstrable lesion or a disease that satisfies established neurological diagnostic criteria(1).

Pain serves to protect the body against (more) potential tissue injury, to assist healing and is therefore useful for survival(15). Nociceptive stimuli can produce simple, unconscious motor reflexes (like withdrawal reaction). The nociceptive or pain threshold is the minimum amount of painful stimulation that provokes this withdrawal reflex. These reflexes are controlled in the spinal cord and do not require consciousness. Sudden acute pain is of short duration and the body's reaction is quick. Depending on the severity of tissue injury the duration of acute pain does not outlast the tissue healing process(7). Acute recurrent pain has a definable cause, leading to repeated periods of pain(15). Prolonged pain is often caused by inflammation with additional hyperalgesia (increased response to a painful stimulus) and allodynia (pain due to a stimulus which does not normally provoke pain).

However, pain may continue with or without an initial cause, leading to (temporal onset of) chronic pain(7, 15). Chronic pain may have long-term consequences, leading to disturbances in endocrine and autonomic functions(7).

Animals in pain often change their behavior. The animal learns from the experience to prevent recurrence of the event(15). Most animals which are in pain reduce their activity level, to prevent hyperalgesia and to promote tissue repair(7). Mental alertness is often decreased, the eyes appear dull and distant and the animal may show no interest in food or water(19). But some animals show hyperactivity and are pacing, weight shifting and changing position in attempt to avoid the pain(7, 15). Scratching, shaking, biting, auto-mutilation, and aggressiveness or submissiveness toward co-species or humans has also been observed. Altered autonomic functions (sympathetic nervous system) which may be observed are elevated respiratory and heart rate, hypertension, salivation, sweating and mydriasis(7, 19). Elevated concentrations of plasma cortisol or catecholamines (e.g. epinephrine, norepinephrine) or in endogenous opioids (e.g. endorphin) may be measured.

The behavioral aspects and autonomic changes of acute pain may continue in animals with chronic pain. They may still be lame, hypoactive, depressed and aggressive or submissive. But also signs of stress or discomfort may develop, such as tremors, shivering, grimacing and stereotypic behavior (e.g. head shaking, scratching, licking, kicking). On the long term weight loss (as a result of loss of appetite) or overeating, incontinence, disturbed sleep-wake cycles and loss of interest in grooming, reproduction and playing are reported. If untreated, chronic pain can lead to abnormalities in endocrine function and immune-suppression, leading to increased susceptibility to infection, systemic illness and stress-related disorders(7).
These behavioral changes are used on daily basis to notice the presence of pain in animals, but don't give an indication of the severity, source, type and progression of the pain. While these points are particularly relevant in a clinical setting for patients monitoring, reaction to therapy (analgesia) and further decision-making. Furthermore, the expression of pain through behavior has some limitations. For example, horses in terminal stages of abdominal disorders (colic) can be severely depressed and show very few signs of pain, or horses who underwent major surgery are not always able to express pain(3). The behavioral changes which are associated with pain are also influenced by individual (character and learned experience) and breed. Animals which were used to be bred for 'adverse conditions' (cold-blood horses, e.g. Friesian or Shetland ponies) are likely to show less pain than warm-blooded horses (e.g. Thoroughbreds)(19).

Head pain in horses

In the horse, there are many conditions that can lead to pain to the head. For example, ear pain, trauma (e.g. jaw-fractures, wounds), eye-problems (e.g. uveitis, cornea-ulcers, injuries), tooth-problems (with secondary sinusitis) or surgery. Some of these disorders are acute and others more chronic, leading to all kinds (nociceptive, neuropathic) and gradations of pain.
The horse its expression of the head pain can vary. Additional to the described signs in the chapter above, there are several behaviors that may be quite specific for head pain. For example, dental pain, can be difficult to recognize. The hidden nature of disorders causing dental pain makes recognition complicated and the problems are often unnoticed, until obvious signs of discomfort are seen. Abnormal bit behavior, behavioral problems during work, drooling of saliva and altered/ slow eating and anorexia with consequently effects on body condition are commonly found indicators for dental pain(3, 19). To the horse owner, quidding (dropping partly chewed food) and food pocketing or pouching in the cheeks are often a clear sign of dental problems. When the horse suffers from acute eye problems, it often shows swollen conjunctiva, excessive lacrimation and/or discharge or corneal edema. Because of the, sometimes severe, pain the horse shows blepharospasm, miosis and photo-sensitivity. In more chronic cases, these symptoms may be present in a lesser extent, but can lead to a horse that expresses more general chronic pain as described above.
Severe (chronic) pain to the head can evolve from headshaking to a lowered head, head pressing and/or eventually extreme depression. Headshaking in horses is generally suggestive for (neuropathic) head pain (including pain to the temporo-mandibular system) and horizontal headshaking may be indicative for ear pain. Head pain often provokes snorting and restlessness(3, 19). But since headshaking can be caused by many different causes, it can become an established behavior. It has a high individual variation and is therefore not a reliable indicator for head pain(3).

Pain assessment

To provide every animal with individual tailored pain management the recognition of pain is very important. Furthermore, the assessment of pain in animals is also very important for clinical evaluation and decision-making(3, 5). Unfortunately pain in animals is very difficult and hard to measure, unlike in verbal humans.

Humans, excluding those unable to self-express or with impaired verbal skills (e.g. babies, geriatrics or disabled), are able to verbally give an indication of the severity of their pain. In human medicine a large number of pain scales are developed and used in hospitals, both for verbal and non-verbal children and verbal and non-verbal adults.

Some scales are based on behavioral parameters to measure the unpleasant experience, mostly in non-verbal (critically-ill) persons. In, for example, the Checklist of Nonverbal Pain Indicators (CNPI), and the Facial Action Coding System (FACS) observers try to interpret the amount of pain the person is in(2, 4, 9, 11).

Other scales are based on the patient's own opinion, in for example the Visual Analogue Scale (Figure 2). In the Numerical Rating Scale the patient is asked to indicate the intensity of pain by reporting a number from 0 to 10(4). For children the Faces Pain Scale (FPS) is a commonly used measurement (Figure 1), in which children are asked to point at drawings of painful faces which subsequently indicates the amount of pain a child is in(11).

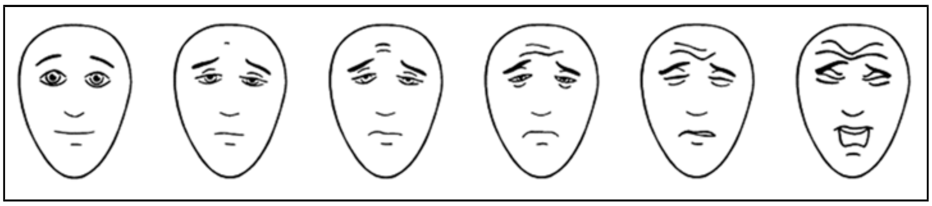


Figure 1 - Faces Pain Scale: from left to right amount of pain scored 0-2-4-6-8-10 (or 0-1-2-3-4-5)(11).

These kinds of direct measurement in animals is impossible and animal pain is only noticed by other indicators of the unpleasant mental state such as behavioral and physiological changes(15). In recent years, in veterinary medicine multi-dimensional pain scales are described for animals, resulting in that not only (obvious) severe pain is recognized, but also symptoms of mild to moderate pain can be observed(3, 5, 8, 10, 14, 18). Since pain is very difficult to measure, the scales must be reliable, repeatable and valid. Many of the pain scales for animals are designed for pain assessment for one particular disease, e.g. post-abdominal surgery, orthopedic pain, chronic pain(5, 10, 12, 16, 17). It is clear that different species need their own pain scales, because each species has a different way to express pain. Furthermore, interpretation of pain in all animals depends on understanding its normal behavior and the same applies for the behavior of horses(19).

A subjective animal pain scale system is the Visual Analogue Scale (VAS; Figure 2). The VAS is a 10 cm line with two end points: the left end equalizes no pain (=0) and the right end unbearable pain (=10). Observers of an animal place a mark on the line corresponding to their interpretation of the patient's pain intensity. The VAS appears to be highly subjective and unreliable when used by persons who are not trained to observe pain (e.g. owners), and because of the low inter-observer reliability it has a poor repeatability(3, 12) .

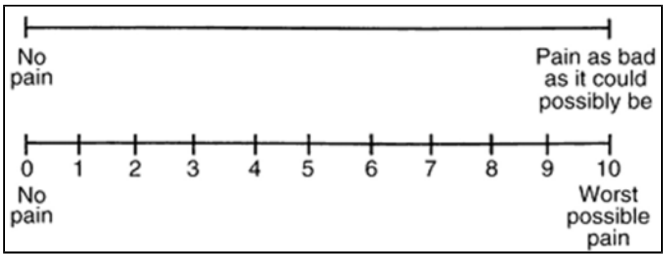


Figure 2 – Visual Analog Scales

More objective systems are based on multifactorial behavioral or physiological parameters, for instance the Post Abdominal Surgery Pain Assessment Scale (PASPAS), the Composite Pain Scale (CPS) and the Rat Grimace Pain Scale(5, 10, 12, 14, 18).

Composite Pain Scale (CPS)

The CPS is a composite numerical rating scale integrating physiological data (heart rate, respiratory rate, etc) and behavior such as responses to stimuli and spontaneous (interactive) behavior(14). All measurements are rated from 0 (normal; no pain) to 3 (behavior or physiologic changes due to presence of pain). The sum of all these scores leads to a total score between 0 (representing no signs of the mentioned criteria) and a certain maximum (representing maximum score of criteria)(5, 14). The scoring system showed in both studies a (very) high reproducibility and repeatability (low inter-observer variability) when used in horses with acute synovitis or post-surgery abdominal pain, which made it a potential promising tool to asses pain(5, 14). With this scoring system, the clinician has an objective (day- to- day) pain evaluation tool to assess the pain status of horses with acute surgical and nonsurgical painful conditions. Using the CPS the clinician is able to discriminate the amount of pain objectively between horses and also assess the effect of analgesic treatment(14). Different persons are able to score the pain whether or not they know the history and/ or current treatment of the case.

Facial expression pain scales

Humans and animals are capable to express emotions including pain through facial expression. In humans grimacing, frowning, and a wrinkled forehead and teary eyes/crying are indicative for pain. The Facial Action Coding System (FACS) is developed to score pain in the critical-ill, non-communicative (due to tracheal intubation, unconsciousness, sedation or paralysis) human patients. It identifies and scores facial muscle actions (action units) and specific patterns during an emotional response, such as pain. The system has been shown to be highly reliable(2).
Since no studies on facial expressions other than in humans were performed, lately several studies focused on facial expressions of pain in animals(13, 18).

Figure 3: A pain face in human FACS. Left: neutral expression. Right: pain expression (2)

For example, the mouse grimace scale (MGS) and the Rat Grimace Scale (RGS) are compositions of 'no-pain' (baseline) and 'pain' photographic coding systems, as seen in Figure 4. The scales are used for pain assessment in the laboratory mice and rats for veterinary care and drug development. Orbital tightening, nose and cheek bulge, ear position and whisker changes are the 'pain face' action units scored in the MGS and RGS. It is a standardized behavioral coding system with high accuracy, reliability and validity(13, 18).

Horses have many facial expressions or behavioral changes to show for example emotions like pain, fear or relaxation. A fixed stare, dilated nostrils and a clenched jaw are general facial expressions of fear, but can also be seen in (chronic) pain. A lowered head carriage is often associated with depression due to acute or chronic, severe and often unrelenting pain(3).

Recently, a larger interest in facial expression of pain in horses has led to development of facial expression pain scales for horses(6). In these studies, researchers observed different facial actions to identify pain. Stiffly backward ears, orbital tightening, tension above the eye, strained mouth and chewing muscles, pronounced chin, strained nostrils and flattening of the profile are element which were scored. Example of two (out of total six) Facial Action Units (FAUs) of the Horse Grimace Scale (HGS) are shown in Figure 5. The HGS showed a high inter-observer reliability and a reasonable high degree of accuracy (73.3%). According to the authors darker colored horses were more difficult to score than lighter colored horses. FAUs such as orbital tightening, tension above the eye, strained mouth and chewing muscles were more difficult to see in the pictures(6).

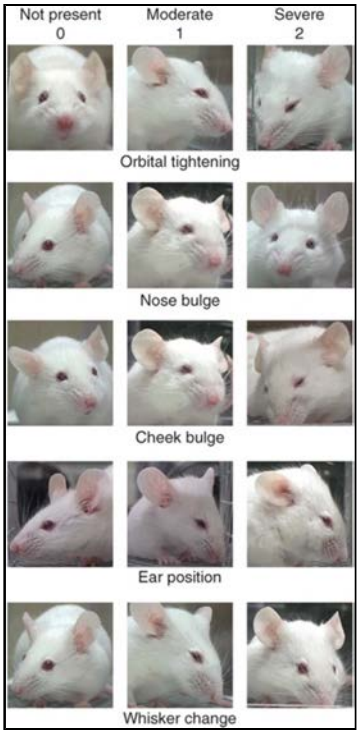


Figure 4: 'Pain face' action units scored in the Mouse Grimace Scale(13)

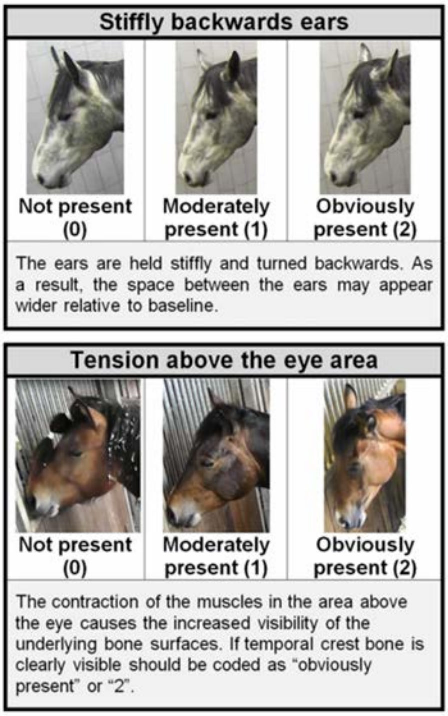


Figure 5: Two (out of six) Facial Action Units (FAUs) of the Horse Grimace Scale (HGS)(6)

Aim of the study

At the Equine Health Department of the Faculty of Veterinary Medicine in Utrecht horses with problems to the head often undergo standing or recumbent surgery. These are sometimes less-invasive or invasive procedures, for example a surgical removal of the eye or the creation of boneflaps giving access to repulsion or extraction of teeth, fractures of mandibular/maxilla. Most of the time, regardless of the nature and invasiveness of the surgical procedure, all horses are administered NSAIDs (Meloxicam p.o.) pre- and post-surgery. The same applies for horses with eye problems (e.g. uveitis, trauma), which also most of the time receive an NSAID (Meloxicam), or sometimes no analgesia at all. But the pain caused by the different procedures might not be comparable and a more individually tailored analgesia may be appropriate in contrast to the current therapy consisting of NSAIDs. To give the horse a more tailored analgesia it is best to monitor the pain in these patients with facial pain. This leads to the following aim of this study:

The construction and internal validation of objective pain scales and comparison of different pain scales in horses with facial pain.

In this study 'facial pain' is defined as pain caused by pain to the eye (or eyes, causes by e.g. uveitis, glaucoma or trauma) or other causes of pain, for example dental pain, sinusitis, surgery or a jaw fracture.

The hypothesis is that the Composite Pain Scale (CPS) and the Facial Expression Pain Scale (FEPS) are reliable objective scoring systems to monitor facial pain in horses. Therefore, it will improve equine well-being which subsequently leads to a faster recovery of the horse.

Materials & Methods

Eight horses of various breeds (3-19 years old) with different painful conditions to the head admitted to the Equine Health Department of the Faculty of Veterinary Medicine in Utrecht between 19/08/2013 and 6/10/2013 were used for this study. The control group consisted of eight horses owned by the Department of Equine Sciences, seven mares and one gelding (4-15 years old, various breeds) which normally were used for education. All horses underwent regular physical examination and basic dental inspection and only healthy (pain-free) horses were included in the control group. Cases and controls are described in Table 1 below.

Horse	Diagnosis	Location	Surgery	Breed	Age	♀/♂
Heel01	Alveolitis	106	Teeth extraction	Friesian	3Y, 4M	♀
Heel03 **Heel05** **Heel08**	Chronic eye problem	Unilateral	Enucleation OS Enucleation OS Enucleation OD	Friesian Thoroughbred Friesian	18Y, 3M 13Y, 8M 12Y, 3M	♀ ♂ ♀
Heel02	Glaucoom, ERU	OS	-	NRPS	19Y, 4M	♀
Heel07	Jaw-fracture	Maxilla extra-articulair	Cerclage maxilla	KWPN	-	♀
Heel06	Sinusitis, periostitis	Sinus frontalis + maxillaris	2x: Sinus-trepanation by frontonasal bone-flaps, and 2 flushing systems	Trakehner	8Y, 5M	♀
Heel04	Uveitis	Bilateral	-	KWPN	3Y, 4M	♀
Con01 **Con02** **Con03** **Con04** **Con05** **Con06** **Con07** **Con08**				KWPN KWPN KWPN Friesian KWPN KWPN KWPN Friesian	8Y 7Y 18Y 7Y, 5M 10Y, 6M 15Y 4Y 13Y,7M	♀ ♀ ♀ ♀ ♀ ♀ ♀ ♂

Table 1 : Cases and controls used in this study.

Horses which underwent surgery received analgesia (local anesthesia and/or opiates and/or NSAIDs both administered systemically) together with (general) anesthesia. Post-surgery they received Meloxicam (Metacam p.o., 1 dd) for at least three days. Horse 'Heel06' received Meloxicam the whole time and the horse with bilateral uveitis (Heel04) was given Atropine (topical intra-ocular administration) 10 times daily.

Three pain scales were used in this study: the Composite Pain Scale (CPS), the Facial Expression Pain Scale (FEPS) and the Visual Analogue Scale (VAS) details present in appendix 1,2 and 3 respectively. The CPS used in this study is an adjusted scale based on the CPS described by Bussières et al(5). Several elements (laying down/ rolling, tail flicking and pain sounds) were added. This CPS consists of physiological data (heart rate, respiratory rate, temperature, sweating and digestive sounds), responses to stimuli (reaction to observer, reaction to palpation painful area) and spontaneous behavior (posture, laying down, tail flicking, pawing, head movements). The FEPS used in this study was created at Equine Department of the University and used in previous research.
In the CPS and FEPS the parameters were scored by composite numerical rating, resulting in a total maximum score out of 42 (CPS) or 18 (FEPS). In the VAS score a location on a continuous scale of 0-

10 was given by the treating vet at the Equine Clinic. Scoring of CPS and FEPS were performed independently at the same time by two observers, not blinded for the reason of admittance or treatment of the case. Treatment of the cases was adjusted due to the normal clinical process and was not dependent on outcome of the pain score measured for this study.
Scoring of the CPS and FEPS was performed at two moments (morning and afternoon) during three days following admittance to monitor the pain and notice the changes in pain in time. Horses treated conservatively where scored at T=0, T=1st morning and T=2nd morning. The cases which underwent surgery were scored at T=0 pre- surgery, T=0 post-surgery (4-5 hours post-surgery), T= 1st morning, T=1st afternoon, T=2nd morning and T=2nd afternoon post-surgery.

Figure 6: Standard scoring environment used in this study

During pain scoring all horses (cases and controls) were placed in the same stable as seen in the photo in Figure 6, to minimize any environmental influences on the results. The CPS was scored during five minutes directly after entering this stable and the FEPS during one minute after scoring CPS.
In addition, prior to moving of the horse to the standard stable, the FEPS was also scored during one minute in the stable where the cases resided during hospitalization. Reason to do this was to find out whether the horse expressed more or less pain in a familiar environment. This was also done with the control horses. During these scoring sessions video- and photo material was collected, which was used after owners consent.

Methods
The CPS and FEPS scoring systems in horses with facial pain are validated using several criteria:

- Repeatability/reproducibility of the pain scoring systems. The inter-observer-reliability of observer 1 with observer 2 was analyzed using Spearman's rho correlation analysis. This determined the degree of reproducibility of measurements of the two observers in the same circumstances.
- Specificity en sensitivity of pain scales for facial pain were calculated by hand. The mean scores of healthy horses (control group) were compared with the cases. Also the mean FEPS (standard stable) and the FEPS score in the own stable were compared between cases and controls.
  Specificity and sensitivity were calculated with the scores at T=1a post-surgery. The

sensitivity and specificity were calculated with a CPS score range between ≤5 (negative) and >5 (positive). For the FEPS and FEPSos a range of ≤3 (negative) and >3 (positive) was used.

- A Mann Whitney U-test was used to determine the differences in pain scores between cases and controls and differences between the scales.
- Correlation between the different pain scales (CPS, FEPS, VAS) and comparison to the golden standard (VAS score) were determined using Spearman's rho. The mean score of the two observers CPS, FEPS and FEPSos were used.
- The effects of surgery (or analgesia) on total pain score over time.

Data and statistical analysis is performed using IBM SPSS statistics 20 and Microsoft Excel 2010. Significance was set at $p < 0.05$.

Results

Reproducibility of the pain scoring systems.

In Table 2 and Figures 7,8 and 9 the strength of the linear association between the two independent observers is shown. Table 2 describes the correlation coefficient (r) between the two observers for all scales. The correlation coefficient between the two observers was highly significant ($p<0.01$) for all pain scales.

The black line drawn in Figures 7,8 and 9 shows the perfect linear association if both observers would consequently had given the same pain score to a horse. The colored line is the line of the tested scoring system: respectively CPS, FEPS and FEPS in the own stable (FEPSos). The correlation coefficients between observers scores showed to be very strong for the CPS and FEPS score and strong for the FEPS.

	Correlation coefficient (r)	R^2	n	Figure
CPS	0.921**	0.84	35	7
FEPS	0.823**	0.67	35	8
FEPS os	0.911**	0.83	27	9

*Table 2 : Inter-observer association of pain scales. *: p=0.05-0.01, ** : p=0.01 – 0.001.*

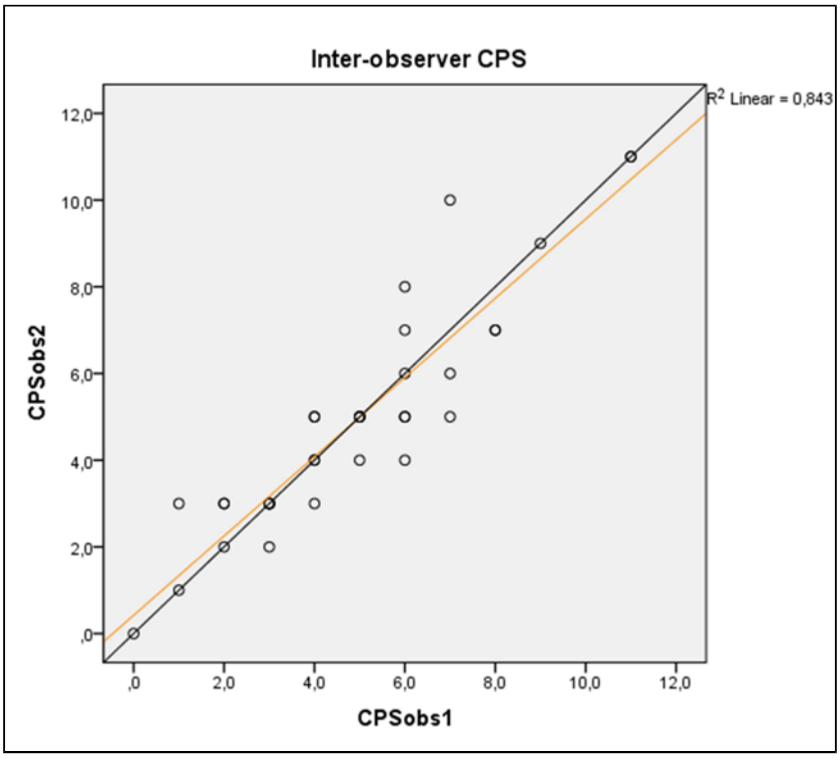


*Figure 7: Inter-observer association CPS. CPSobs= score observer 1 or 2, n=35 all observations performed in duplo, r= 0.921**, p<0.05*

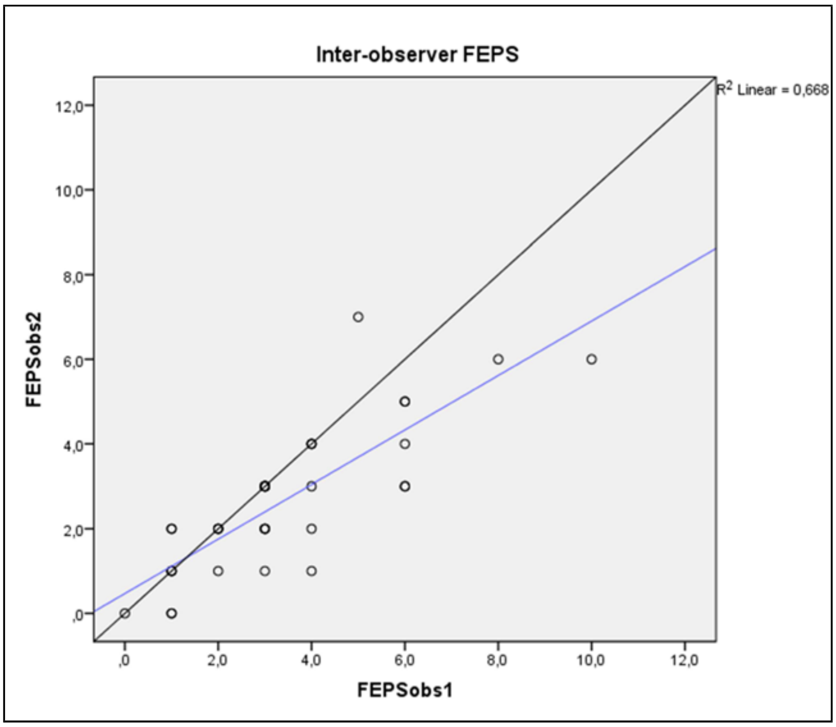


*Figure 8 : Inter-observer association FEPS. FEPSobs= score observer 1 or 2, n=35 all observations performed in duplo, r= 0.823**, p<0.05*

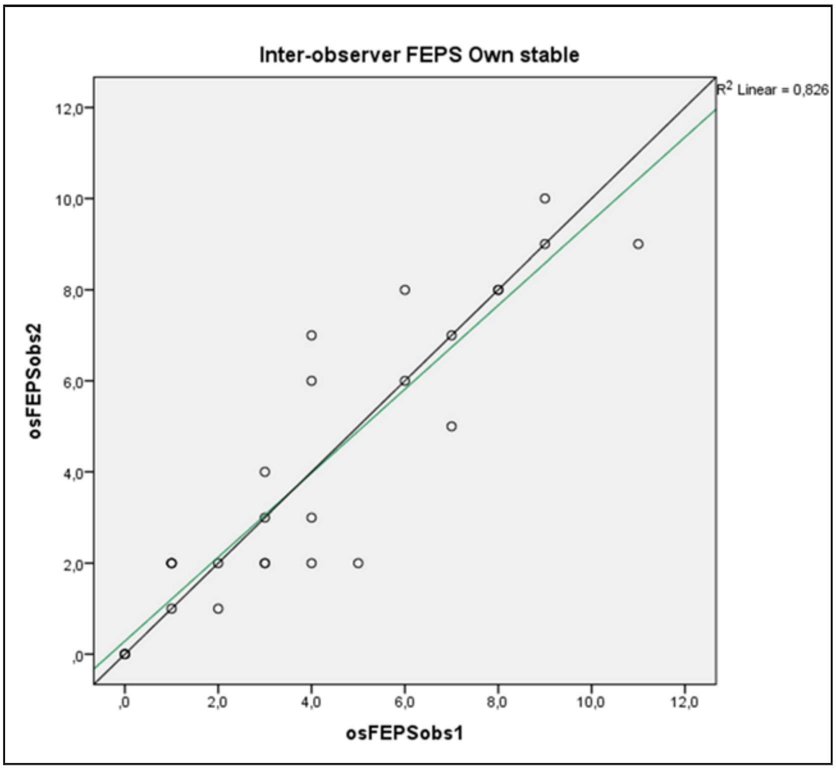


*Figure 9 : Inter-observer association FEPS own stable, osFEPSobs= score observer 1 or 2, n=27 all observations performed in duplo, r= 0.911**, p<0.05*

Specificity and sensitivity of the pain scoring systems for facial pain.
To identify the specificity and sensitivity of the scoring systems for facial pain, the pain score of all scales between cases and controls are compared, as described in Table 3.

	Sensitivity	Specificity	PPV	NPV	n cases
CPS	42.86 %	75.00 %	60.00 %	60.00 %	8
FEPS	42.86 %	87.50 %	75.00 %	64.00 %	8
FEPSos	99.99 %	99.99 %	100 %	100 %	4

Table 3: Sensitivity and specificity of pain scales at T=1a. PPV = positive predictive value. NPV= negative predictive value.

As seen in the boxplot in Figure 10 the distribution of the mean CPS and mean FEPS is approximately the same across cases and controls. Distribution of FEPSos is not the same between the subgroups and, as described in Table 4, a significant difference between cases and controls existed. In Figure 10 horse number 33 is out of range of the average score, this is a control horse which scored relatively high (5.5) in the FEPS.

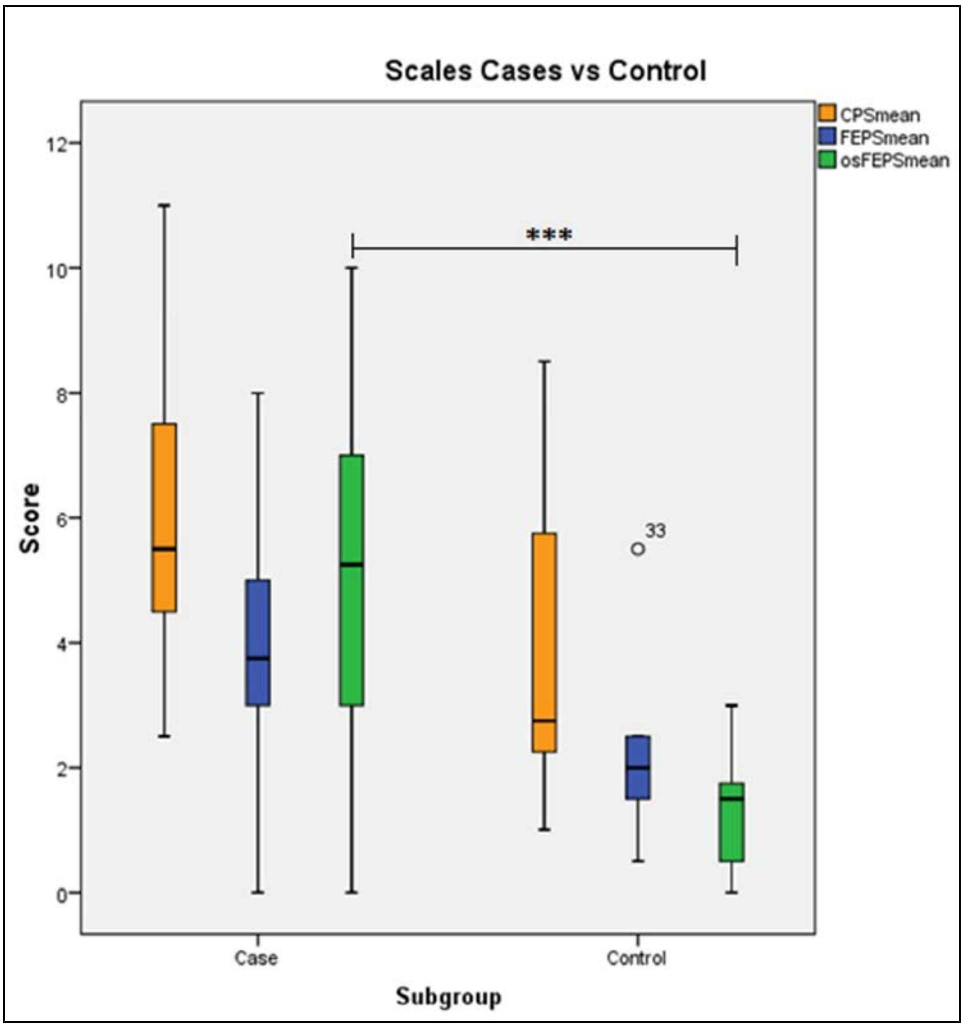


Figure 10: Distribution of pain scales, mean score cases vs. controls.
Cases (n=18) : CPSmean: 25%-quartile=4.5, 75%-quartile=7.5, median=5.5, minimum=2.5, maximum=11.0. FEPSmean: 25%-quartile=3.0, 75%-quartile=5.0, median=3.75, minimum=0.0, maximum=8.0. osFEPSmean: 25%-quartile=3.0, 75%-quartile=7.0, Median= 5.25, minimum=0.0, maximum=10.
Controls (n=8) : CPSmean: 25%-quartile=2.25, 75%-quartile=5.75, median=2.75, minimum=1.0, maximum=8.5. FEPSmean: 25%-quartile=1.5, 75%-quartile=2.5, median=2.0, minimum=5.0, maximum=5.5, with outlier nr. 33. osFEPSmean: 25%-quartile=0.5, 75%-quartile=1.75, median=1.5, minimum=0.0, maximum=3.0.

	p-value (<0.05)	n cases
CPS	0.122	27
FEPS	0.122	27
FEPSos	<0.001*	19

*Table 4 : Specificity of pain scales, mean score cases vs controls, *:p= 0.05-0.01, **:p= 0.01 – 0.001*

Correlation of pain scoring systems.

In Table 5 the correlation between the different pain scales (CPS, FEPS, VAS) and comparison to the golden standard (VAS score) is shown.

The VAS score (collected at T=0) is compared to the CPS, FEPS and the FEPSos at T=0. There is no significant correlation between the VAS score and the tested pain scales. As seen in Table 5 CPS, FEPS and FEPSos correlate and are significant.

	FEPS	FEPSos	N
CPS	0.598**	0.464*	35, 26
FEPS	-	0.678**	26

*Table 5 : Correlation coefficient between pain scales. *:p= 0.05-0.01, **:p= 0.01 – 0.001*

	VAS (T=0)	N
CPS (T=0)	0.000	8
FEPS (T=0)	0.020	8
FEPSos (T=0)	0.441	8

Table 6 : Correlation coefficient of VAS with pain scales at T=0

The effects of surgery on total pain score.

In Figures 11 and 12 the CPS, FEPS and FEPSos timeline are shown.

In the timelines of all scoring systems most cases show an increase in pain score at 4/5 hours or 24 hours post-surgery. The pain score gradually dropped after 36 hours post-surgery, with exception of one case (Heel03) and another case (Heel05). They both scored a few points higher (at 36 or 48 hours post-surgery) relatively to their previous score.

Case Heel06 underwent two surgical procedures, with one month interval. This horse was scored at -48 hours pre second surgery. The second surgery was performed 3,5 weeks after the first surgery, after which the horse was still painful. The horse could not be scored more moments post second surgery because it was moved to the isolation area of the clinic because of a MRSA wound- infection. Some cases are not scored in the own stable (FEPSos) every scoring moment (Heel01, Heel03, Heel08), because they did not have a ‘own stable’ (in case of scoring at arrival, T=0) or were moved to another stable.

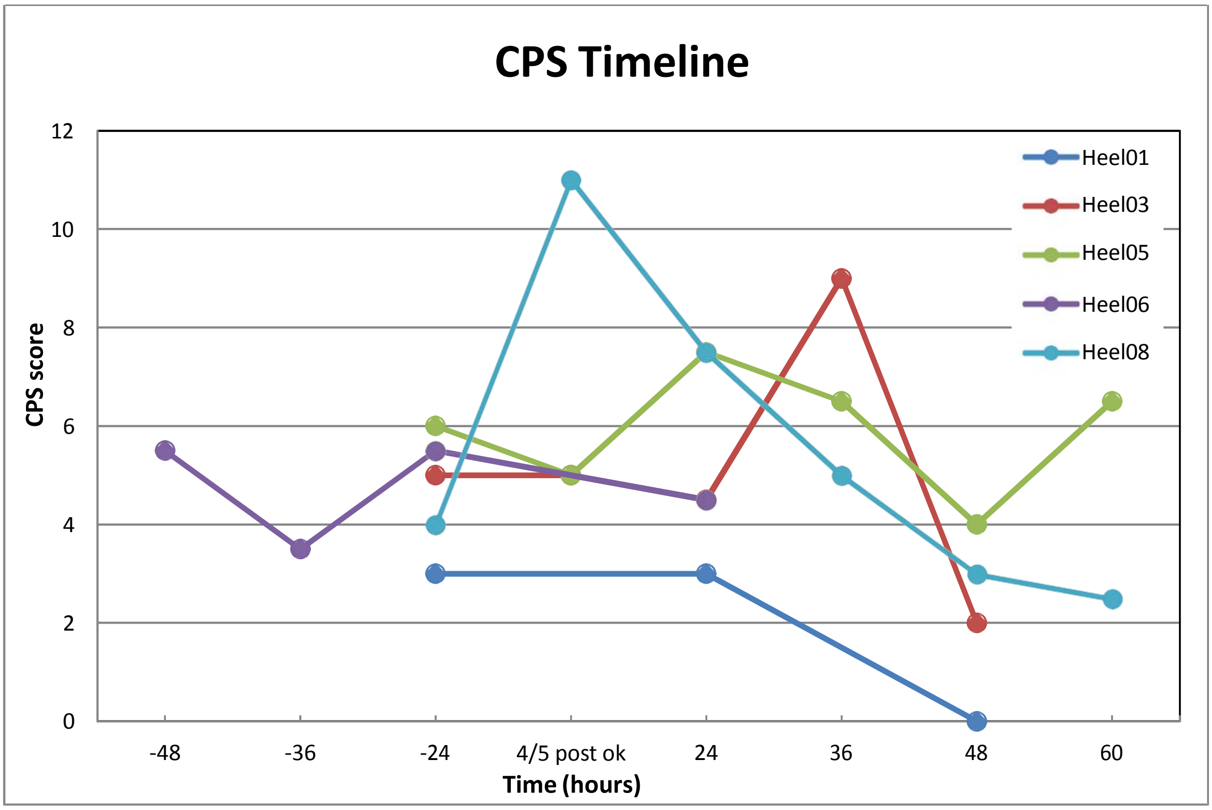


Figure 11 : CPS timeline (mean score) pre- and post-surgery for n = 5 cases

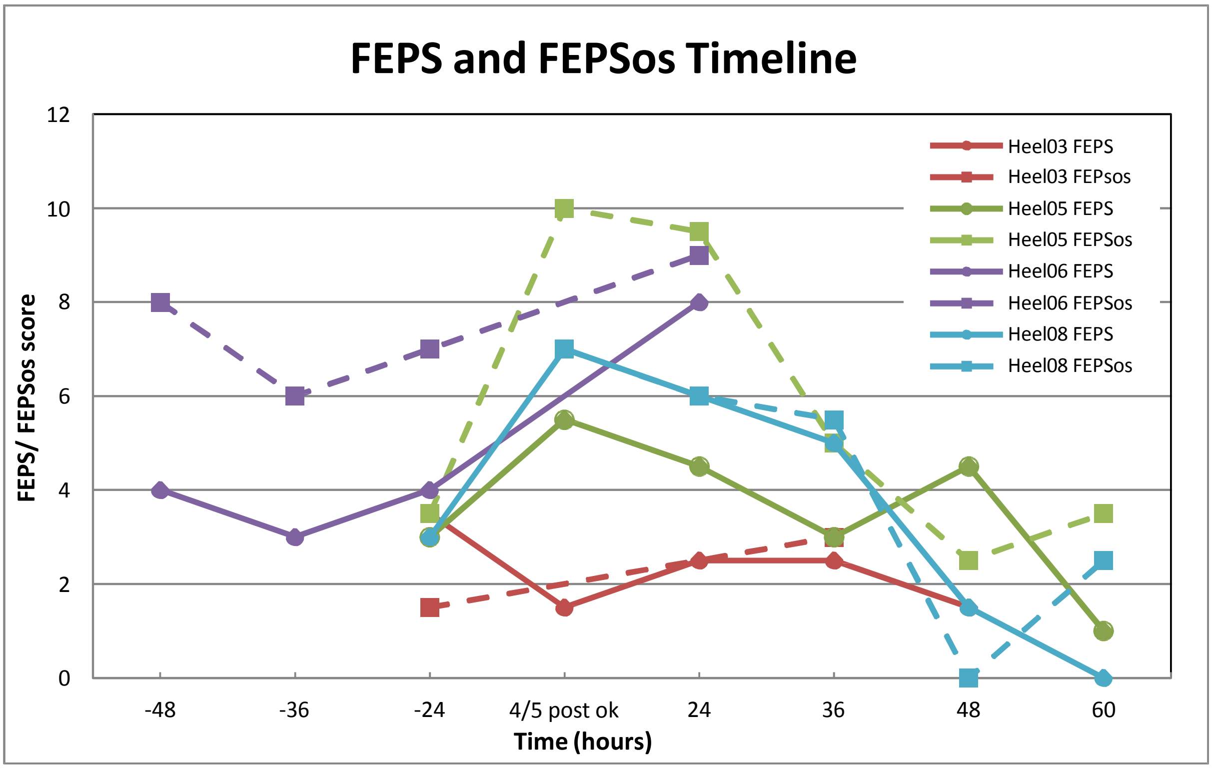


Figure 12 : Comparison mean score between FEPS and FEPSos timeline pre- and post-surgery for n = 4 cases. After 4/5 post-ok all horses received analgesia. Case Heel06 received analgesia the whole time.

Discussion

An ideal pain scoring system is linear, weighted, sensitive to pain-type, breed-specific, objective (not dependent on observer) and clearly described to avoid misinterpretation(3).
To determine these aspects different statistical analysis were applied to validate the two objective pain scoring systems for facial pain in horses. Both pain scoring systems are highly objective and clearly described. Combining all results the conclusion based on these data is that the FEPS scoring system to be most suitable for horses with facial pain and is most reliable when performed in a familiar area (own stable). The FEPSos has a good repeatability and a high sensitivity and specificity.

The overall inter-observer reliability within CPS, FEPS and FEPSos was high. A high inter-observer reliability is seen in lots of other studies using multidimensional numeric rating scales(5, 6, 14). For example, in the study of van Loon et al. (2010) the weighted kappa correlation coefficient of the CPS was κ= 0.81 (very high) and the study of Dalla Costa et al. (2014) showed more than 80% agreement between the observers(6, 14). In this study, the two observers generally gave approximately the same scores at a certain scoring moment resulting in a (very) strong correlation. There was a high correlation between both observers in the CPS (R^2=0.84) and FEPSos (R^2=0.83) and a reasonable correlation (R^2=0.67) between both observers in the FEPS. This meant the pain scales showed a good repeatability, which make the scales reliable. It also makes the pain scales well-suited for the clinical setting, since different observers are able to score the horse without affecting the reliability of the total pain score.
As seen in Figure 8, there were a number of FEPS scoring moments when the observers differed from each other in the total score, with a maximum dissimilarity of four points. This relatively large difference in the given points could have had multiple causes, which will be discussed later. In the end, this relatively 'large' difference resulted in a lower correlation within the FEPS.

For the CPS, generally a low pain score in the controls was found. Together with the relatively low sensitivity for facial pain it makes the CPS not suitable to use for head pain prior to an operation, despite the high inter-observer reliability. No big differences (>3 points) in the CPS score between acute cases (Heel02,03,07,08) or more chronic cases (Heel05,06) were seen pre-surgery, therefore the duration of pain does not seem to influence the initial CPS score.
In Figure 10 the FEPS seemed to show a difference in pain score between cases and controls, but this difference was not significant. Control number 33 was a horse, which scored relatively high in the FEPS. This horse scored a total mean of 5.5 points in the chapter eyelids, nostrils, corners mouth/ lips and muscle tone of the head. Since in the CPS heart rate, respiratory rate, reaction to observer and reaction to palpation also scored high in this horse, the high facial score could be anxiety- or stress-related. Since the horse was not in pain (control), this is a false positive control.
The FEPSos was able to significantly discriminate facial pain between cases and controls. The horses seemed to express more pain when they reside in a familiar environment. For example: in the photos in Figure 13 is shown an example of the facial expression of case Heel05, 4/5 hours post-ok in its own stable (left) and the standard scoring environment (right), with ± 5 minutes interval due to the walk through the clinic. On the picture on the left is seen that the horse shows tightening of the eyelids, keeps its head tilted to the non-painful side, with ears positioned backwards with no interest in the environment. In the picture on the right a more alert horse is seen, with open eyes and more

focus on the environment. This horse had a FEPS score of 5.5/18 in the standard scoring box and 10/18 in its own stable, which was a big difference in total score.

Figure 13: Case Heel05, 4/5 hours post-ok with ± 5 minutes interval. Left: in own stable and a FEPSos mean pain score of 10/18. Right: in standard scoring stable with a mean FEPS pain score of 5.5/18.

The moving and guidance through the clinic somehow caused a lower pain score in the test stable. It is likely that this is caused by stress (excitement or fear) due to the movement which may alter their behavior and mask signs of pain(19). As described earlier, hiding pain or injury prevents standing out to predators(7). Maybe the horse 'feels' less pain because of higher adrenaline levels due to anxiety caused by the strange environment or absence of other horses, or anticipation for the procedures to come. Or the horse gets distracted from the pain because it focuses on other things than pain. However, the reduction of pain expression does not necessarily mean that the horse suffers less, but it may as well be.
But the question remains when the horse thinks the environment is familiar, since they usually did not reside there longer than one or two days (admittance one day pre-surgery). However, this was contradicted by horse Heel06, which was already in clinic for three weeks (in the same stable). But to find out the environmental influence, more cases should be admitted and scored earlier (than one day before surgery) to the hospital for example as was done by Della Costa et al. (2014)(6). To control for any possible effects of stress, related to being a novel environment or separated from peers, the horses were admitted to the clinic and placed in the scoring/ monitoring box two days prior to the beginning of the study to acclimatize. Price et al. (2003) started the behavioral assessment at least 24 hours after admittance to the hospital(16). In both studies the horses were deprived of food (overnight) and the horses were scored in the same stable as they resided (they were not moved to a standard scoring stable)(6, 16). In previous studies in which pain associated with post-abdominal-surgery was assessed, there was no time for the horses to get used to the environment because of the emergency circumstances(10, 14). Except for the study of Della Costa et al., in which stallions admitted for castration were used, the cases in the mentioned studies above were in a certain degree of (abdominal, soft tissue, orthopedic) pain(6, 10, 14, 16). All of these studies have their limitations and it remains a question whether how much impact (the combination of) accustoming to the environment, deprivation of food and existing pain has on the pain score.

Based on only 4 patients: the sensitivity of the CPS and FEPS for facial pain pre-surgery were low, which meant that the scoring systems were not able to discriminate painful animals from non-painful horses and a high rate of false negative outcomes were seen. But the specificity of both systems on contrary, are high: there were a few false positive outcomes. The FEPSos had a very high sensitivity (99%) and specificity (99%) for facial pain. The positive predictive value and negative predictive value for FEPSos were both 100%, which meant that all painful horses scored >3 points and all control horses scored ≤3 points. This resembled a perfect test and is very promising. Though this is not reliable, since only four patients and eight controls were used to calculate these outcomes. The outcomes could be influenced since the values which defined pain (positive) or no pain (negative) used here were chosen by the author. To calculate more reliable sensitivity and specificity for the FEPSos, much more horses and controls must be scored and compared.

When the different scoring systems were compared, there was a poor correlation between the subjective (VAS) and objective (CPS, FEPS, FEPSos) pain scoring systems. The pain scales did not correlate with the golden standard (VAS). The question raised if the VAS is a suitable golden standard since several studies found acceptable, but poor inter-observer reliability using subjective pain scales(5, 12). The subjective pain scales were highly influenced by the ability of the observer to recognize pain(5). In this study was assumed that treating vets at the Equine Clinic are (more than) able to recognize pain. The reliability of the VAS score would be higher if the mean score of more than one (treating) vet was used and the repeatability (inter-observer-reliability) of the VAS for facial pain could be calculated. Perhaps there would be an explanation for the low correlation between the scoring systems.

In the CPS timeline and FEPS-FEPSos timeline an effect of surgery on mean pain score was shown. Most cases showed an increase in pain score at 4/5 hours or 24 hours post-surgery. The pain score gradually dropped after 36 hours post-surgery. This decline in (CPS) pain score over days was also seen in a the study of van Loon et al. (2010) in which was found that the CPS is very useful for the recognition of changing pain states after analgesia or surgery(14).
Post-surgery all horses were given NSAIDs (Meloxicam p.o.), which meant all horses were under influence of analgesia post-surgery. Only horse Heel06 was given NSAIDs during the entire period. Probably the overall pain score would be higher if the horses received no analgesia (post-surgery), but this would be ethically unacceptable. A smaller difference would possibly be seen between T0 pre- and T0 post-surgery if the horses received NSAIDs a few days pre-surgery. Though, it is important to recognize that although analgesia post-surgery may give lower pain scores, this does not necessarily mean that the horse received clinically relevant pain relief(19).
The same applied for the effects of anesthesia, which could have effects on the horse pain experience and pain expression. To investigate the impact of anesthesia on the PASPAS score, Graubner et al. used horses undergoing general anesthesia for diagnostic imaging (in addition to the cases) for pain assessment. It appeared that general anesthesia did not have an effect on the total pain index when compared to the control group(10). To rule out effects of anesthesia on pain scoring on CPS and FEPS, an additional control group, which underwent anesthesia but no surgery (e.g. MRI or CT patients) could be included in the study.

A CPS with a combination of behavioral and physiological element was used for the evaluation of visceral pain in horses was used in this study. Behavioral signs are not objective, except when the horse does the behavior for a longer time or more frequently in association with pain(19). Kicking or looking at the abdomen, tail-flicking and repeatedly laying-down are quite specific for abdominal

pain(14, 19). Physiological elements like heart rate could be elevated in a horse in pain, but also for other reasons such as stress, shock, medications, anticipation or other reasons. That is probably the reason why the facial cases score was relatively low in the CPS: highest score in cases was 11/42, and for controls 9/42. As shown in Table 3 and 4 the CPS was not specific and sensitive for facial pain, when compared with controls. As mentioned in the introduction, physiological elements like respiratory and heart rate or behavior like pawing at floor or restlessness can be easily influenced by stress or excitement. This could attribute to higher scores in some cases.
Also most horses showed a slightly tucked up abdomen as a result of the feed deprivation prior to anesthesia and therefore scored often one point at posture in the CPS. Case Heel08 was a horse which scored points on the elements influenced by stress (e.g. pawing, restlessness). In this case a decrease in CPS score in the following days after surgery was seen. This was maybe caused by adaptation to the clinical setting and standard stable with consequently resulted in less excitement or stress and not the reduction of pain. There are some elements (teeth grinding and reaction to palpation of painful area) in the CPS in which facial pain patient seemed to score more often than other elements. For following studies, when developing a scoring system for facial pain it could be advisable to weigh these elements more than other elements.
This study showed that it is not advisable to use only one general scoring system for all types of pain. Ultimately, all elements should be weighed for specificity for certain pain to lead eventually to different validated scoring systems for different types of pain.

Despite the fairly good results, the study had its limitations. First, observer-bias cannot be ruled out, since observers were not blinded for reason of admittance of the cases and status of analgesia of the horse. The same applied for control horses, the observers knew they were pain-free control horses. This bias could be ruled out by developing a system with perfect videos to score the CPS or FEPS, so that observers would be totally blinded. Also it could be that the pain scores were unintentionally influenced by training of the observers, since both observers were unexperienced with scoring using the CPS and FEPS at the beginning of the study.
As described earlier, breed and character had influence on the pain expression of a horse(19). In this study different breeds were used (Friesians, Warmbloods, etc). It could be interesting to test the influences of breed and character on pain score in a follow-up study.
Another point of concern is that horses undergoing diagnosis and treatment are usually in a strange environment and stressed by isolation from other horses, which may alter their behavior and mask signs of pain(19). Therefore, there may also be effects of hospitalization and observer presence on pain score, but these effects are inevitable(14). A solution could be to score the horses at home and blinded for observers. Or it could be possible to hospitalize the horses earlier in the process so they can get used to the surroundings.

An ideal scoring system is clearly described to avoid misinterpretation(3). Both the CPS and FEPS were generally clearly defined. Scoring during five minutes is long enough to determine a pain score. Some points in the CPS are described too extensive, for example the elements "*head movement*" and "*overall appearance*". When scoring overall appearance there were too many extremes: quiet but alert, alert, restless or stupor. There was no 'too slow and not alert' which lots of horses show. Also, lots of horses scored high on the element respiratory rate since it is scored in very little steps (>18 breaths/min gives a high score). But as described earlier, the CPS showed not to be suitable to asses pain in horses with facial pain. It could be possible that it is more applicable in combination

with an FEPS (os) score together.
The description of the FEPS is also clear and easy to use, also for unexperienced persons. But scoring during one minute is on the short side, since some cases showed behaviors outside this one minute (for example teeth grinding or muscle fasciculation).
As described in the study of Dalla Costa et al. (2014), darker horses were more difficult to score than lighter colored ones(6). This was also the case in this study. As seen in the photos in Figure 14 it is more difficult to see for example muscle fasciculation's, tightened eyes or lifted lips in the darker horse in the left picture than the lighter colored horse in the right picture.

Figure 14 : Difference facial expression visibility in darker horse (left) and lighter colored one (right). Eyelids, muscle tone and lips are more difficult to assess.

One element, yawning, which is a element of the FEPS was never seen in all sixteen horses (cases and controls) and could be deleted. Teeth grinding was seen more in horses 4/5 hours post-surgery and in the horses suffering from prolonged (chronic) pain.
In the Horse Grimace Scale (HGS) tension above the eye, a pronounced chin and flattening of the profile were elements that were not scored in this study, but were seen in some of the cases(6). These elements could be an addition to the FEPS. Also some behaviors and indicators were seen in the horses with facial pain, which could be an addition to the FEPS. These were (uni/bilateral) drowsy eyes, enophtalmus, miosis, sucking and licking of the lips, the reaction to eating (food in mouth), head shaking and head pressing against the wall.
In the MGS and RGS five 'action units' were used to score their prominence in still photographs (Figure 4) with aid of computer software (13, 18). This is much more advanced than scoring of subjective observers. It also makes it able to detect weak analgesic effects of analgesia and measure and quantifying pain of moderate duration(18). This modern way of pain scoring with digital video is more complicated because of the horses' size, but recently performed by Dalla Costa et al. A system system with three pictures (not present – obvious pain) of facial expressions (action units) is very useful and also possible to fabricate for horses (see Figure 5). Implication of such an picture-composition in the equine health care could give an relatively quick indication of the pain status of a horse.

Conclusion

The aim of this study was to construct and investigate the possible usefulness of different objective pain scales and to internal validate these under clinical circumstances.
The hypothesis was that the Composite Pain Scale (CPS) and the Facial Expression Pain Scale (FEPS) were reliable objective scoring systems to monitor facial pain in horses.
The conclusion of this study is that a FEPS score in the horse' own stable (FEPSos) is the most reliable and useful pain scoring system to assess pain in horses with facial pain.

Appendix 1.

Composite Pain Scale (CPS)

* Use this pain scale to determine:

- Pain at admitting *T=0*
- If given NSAIDs at university clinic; score after 0.5 - 1 h, *T=0,5/1*

OR - If pain management is unknown/ was administered before arrival at clinic; score after 2-3 h *T=2/3*

- The first and second morning after arrival *T=1st and 2nd morning*.

* Observe the patient for 5 minutes and be aware of sounds produced by the patient.

* Sum the scores to a total Composite Pain score.

Date:
Time:
Observer:
Time frame: T = 0 T = 0,5/1 T = 2/3
T = 1A T=1B T =2A

Patients label

Data	Categories	Score
Physiological data		
Heart rate	24 - 44 beats/min	0
	45 - 52 beats/min	1
	53 - 60 beats/min	2
	> 60 beats/min	3
Respiratory rate	8 - 13 breaths/min	0
	14 - 16 breaths/min	1
	17 - 18 breaths/min	2
	> 18 breaths/min	3
Rectal temperature	36.9 °C - 38.5 °C	0
	36.4 °C - 36.9 °C or 38.5 °C - 39.0 °C	1
	35.9 °C - 36.4 °C or 39.0 °C - 39.5 °C	2
	35.4 °C - 35.9 °C or 39.5 °C - 40.0 °C	3
Digestive sounds	Normal motility	0
	Decreased motility	1
	No motility	2
	Hypermotility or steelband	3
Behavior		
Posture	Quietly standing and/or one hind leg resting, explores environment	0
	Slightly tucked up abdomen, still explores environment (with possible unrest)	1
	Extremely tucked up abdomen, hunched back and/or stretching of body/limbs	2
	Does not stand or for short amounts of time (<1 min), sits on hindquarters	3

Laying down, rolling	Does not lie down or rests lying down Lies down in normal posture, rolls or tries to roll (1-2 times/5 min) Alternates lying down and standing, rolls or tries to roll (>2 times/5 min) Constantly lies in an abnormal position: on its side with stretched limbs, on its back, or does not stop rolling	0 1 2 3
Sweating	No signs of sweating Warm or damp to touch, no sweat or wet spots visible Wet spots visible, no droplets or streams Excessive sweating, may include streams or droplets	0 1 2 3
Tail flicking (do not count flicking to chase off insects)	No tail flicking Occasional tail flicking (1-2 times/5 min) and/or holds tail away from body Frequent tail flicking (3-4 times/5 min), may hold tail away from body Excessive tail flicking (>5 times/5 min)	0 1 2 3
Kicking at abdomen	Quietly standing, no kicking Occasional kicking at abdomen (1-2 times/5 min) Frequent kicking at abdomen (3-4 times/5 min) Excessive kicking at abdomen (>5 times/5 min)	0 1 2 3
Pawing at floor (number of episodes)	Quietly standing, does not paw at floor Occasional pawing at floor (1-2 times/5 min) Frequent pawing at floor (3-4 times/5 min) Excessive pawing at floor (>5 times/5 min)	0 1 2 3
Head movements	No fast movements, head mostly at same height/in same direction Occasional head movements laterally or vertically, looking at flank (1-2 times/5 min) Frequent and fast head movements laterally or vertically, looking at flank (3-4 times/5 min) Excessive head movements, excessive looking at flank (>5 times/5 min), biting at flank (>1 time/5 min)	0 1 2 3
Pain sounds	No audible signs of pain Occasional teeth grinding or moaning (1-2 times/5 min) Frequent teeth grinding or moaning (3-4 times/5 min) Excessive teeth grinding or moaning (>5 times/5 min)	0 1 2 3
Overall appearance, reaction to observer(s)	Quiet but alert, approaches/turns to observer Alert, no reluctance to move, obvious reaction to sounds and/or movements Restless, constantly moving, exaggerated reaction to sounds and/or movements Stupor: the horse is not moving, head is lowered, reluctance to move	0 1 2 3
Reaction to palpation of painful area	No reaction to palpation Mild reaction to palpation Resistance to palpation Violent reaction to (attempt to) palpation	0 1 2 3
Total		.../42

Appendix 2.

Facial Expression Pain Scale (FEPS)

* Observe the patient for 60 seconds and be aware of sounds produced by the patient.
* Sum the scores to a total Facial Pain Expression score.

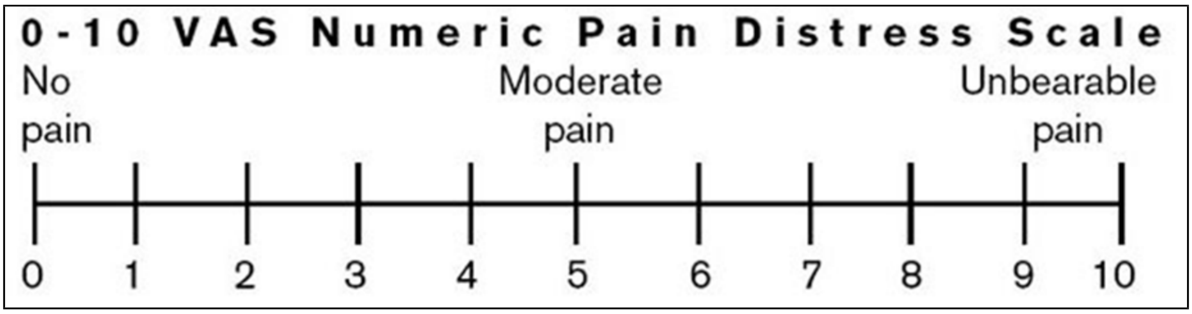
Patients label

Date:
Time:
Observer:
Time frame: T = 0 T = 0,5/1 T = 2/3
T = 1A T=1B T =2A

Data	Categories	Score
Head	Normal head movement/ Interested in environment Less movement No Movement	0 1 2
Eyelids	Opened, sclera can be seen in case of eye/head movement More opened eyes or tightening of eyelids. An edge of the sclera can be seen for 50% of the time Obviously more opened eyes or obvious tightening of eyelids. Sclera can be seen more than 50% of the time	0 1 2
Focus	Focussed on environment Less focussed on environment Not focussed on environment	0 1 2
Nostrils	Relaxed A bit more opened Obviously more opened, nostril flaring and possibly audible breathing	0 1 2
Corners mouth/ Lips	Relaxed Lifted a bit Obvious lifted	0 1 2
Muscle tone head	No fasciculation's Mild fasciculation's Obvious fasciculation's	0 1 2
Flehming and/or Yawn	Not seen Seen	0 2
Teeth grinding and / or moaning	Not been heard Heard	0 2
Ears	Position: Orientation towards sound/ clear response with both ears or ear closest to source Delayed / reduced response to sounds Position: backwards / no response to sounds	0 1 2
Total		.../18

Appendix 3.

Visual Analogue Scale (VAS)



References

1. www.iasp-pain.org

2. Arif-Rahu M and Grap MJ. Facial expression and pain in the critically ill non-communicative patient: State of science review. Intensive and Critical Care Nursing 2010; 26: 343-352.

3. Ashley FH, WatermanPearson AE and Whay HR. Behavioural assessment of pain in horses and donkeys: application to clinical practice and future studies. Equine Vet J 2005; 37: 565-575. many ref.

4. Bijur PE, Latimer CT and Gallagher EJ. Validation of a verbally administered numerical rating scale of acute pain for use in the emergency department. Acad Emerg Med 2003; 10: 390-392.

5. Bussieres G, Jacques C, Lainay O, Beauchamp G, Leblond A, Cadore JL, Desmaizieres LM, Cuvelliez SG and Troncy E. Development of a composite orthopaedic pain scale in horses. Res Vet Sci 2008; 85: 294-306. 37 ref.

6. Dalla Costa E, Minero M, Lebelt D, Stucke D, Canali E and Leach MC. Development of the Horse Grimace Scale (HGS) as a Pain Assessment Tool in Horses Undergoing Routine Castration. PloS one 2014; 9: e92281.

7. Dukes H.H. RWO. Dukes' Physiology of Domestic Animals, Twelfth edition ed. Comstock Publishing Associates, Cornell University Press, Ithaka and London, 2004 pp. 831-839.

8. Dutton DW, Lashnits KJ and Wegner K. Managing severe hoof pain in a horse using multimodal analgesia and a modified composite pain score. Equine Veterinary Education 2009; 21: 37-43. 34 ref.

9. Feldt KS. The checklist of nonverbal pain indicators (CNPI). Pain Manag Nurs 2000; 1: 13-21.

10. Graubner C, Gerber V, Doherr M and Spadavecchia C. Clinical application and reliability of a post abdominal surgery pain assessment scale (PASPAS) in horses. Veterinary Journal 2011; 188: 178-183. 35 ref.

11. Hicks CL, von Baeyer CL, Spafford PA, van Korlaar I and Goodenough B. The Faces Pain Scale – Revised: toward a common metric in pediatric pain measurement. Pain 2001; 93: 173-183.

12. Hielm-Bjorkman AK, Kapatkin AS and Rita HJ. Reliability and validity of a visual analogue scale used by owners to measure chronic pain attributable to osteoarthritis in their dogs. Am J Vet Res 2011; 72: 601-607.

13. Langford DJ, Bailey AL, Chanda ML, Clarke SE, Drummond TE, Echols S, Glick S, Ingrao J, Klassen-Ross T, Lacroix-Fralish ML, Matsumiya L, Sorge RE, Sotocinal SG, Tabaka JM, Wong D, van den Maagdenberg AM, Ferrari MD, Craig KD and Mogil JS. Coding of facial expressions of pain in the laboratory mouse. Nat Methods 2010; 7: 447-449.

14. Loon, J. P. A. M. van, Back W, Hellebrekers LJ and Weeren PR. Application of a composite pain scale to objectively monitor horses with somatic and visceral pain under hospital conditions. Journal of Equine Veterinary Science 2010; 30: 641-649. 26 ref.

15. Molony V and Kent JE. Assessment of acute pain in farm animals using behavioral and physiological measurements. J Anim Sci 1997; 75: 266-272.

16. Price J, Catriona S, Welsh EM and Waran NK. Preliminary evaluation of a behaviour-based system for assessment of post-operative pain in horses following arthroscopic surgery. (Special Issue: Equine Analgesia). Veterinary Anaesthesia and Analgesia 2003; 30: 124-137. many ref.

17. Pritchett LC, Ulibarri C, Roberts MC, Schneider RK and Sellon DC. Identification of potential physiological and behavioral indicators of postoperative pain in horses after exploratory celiotomy for colic. Appl Anim Behav Sci 2003; 80: 31-43. 20 ref.

18. Sotocinal SG, Sorge RE, Zaloum A, Tuttle AH, Martin LJ, Wieskopf JS, Mapplebeck JC, Wei P, Zhan S, Zhang S, McDougall JJ, King OD and Mogil JS. The Rat Grimace Scale: a partially automated method for quantifying pain in the laboratory rat via facial expressions. Mol Pain 2011; 7: 55-8069-7-55.

19. Taylor PM, Pascoe PJ and Mama KR. Diagnosing and treating pain in the horse: where are we today? (Pain management and anesthesia). Veterinary Clinics of North America, Equine Practice 2002; 18: 1-19. 94 ref.