Universiteit Utrecht

Construction of facial expression-based pain scales in foals aged from 14 days to 6 months with acute pain

07-05-2020

M. Bertens

4070151

Supervisor: Dr. J.P.A.M. van Loon

Utrecht University

Veterinary Medicine, Department of Clinical Sciences

Abstract

Pain assessment methods for adult horses have been constructed in the past decade. Foals were never included in these studies, even though they are treated for painful conditions in veterinary clinics. This study describes the construction of a facial expression-based pain scale for foals with acute pain in the age of 14 days to 6 months. The aim of the study was to develop a clinically applicable, reliable and repeatable pain scale. The 'Equine Utrecht University Scale for Facial Assessment of Pain' (EQUUS-FAP) for foals and the 'Horse Pain Face' (HPF) for foals were used to assess the facial pain expression in 32 foals in the age of 14 days to 6 months (10 patients with acute pain, 22 healthy, pain free control foals). 30 seconds long video clips were collected of foals together with the mare in the box. The video clips were randomized and blinded before the observation. Three observers scored the video clips and two of the observers scored all video clips twice. The EQUUS-FAP for foals had a good inter-observer reliability (Cronbach's Alpha (C.A.) 0,90, p<0,001), the HPF for foals had a moderate inter-observer reliability (C.A. 0,71, p<0,001). The intra-observer reliability was good to excellent for both pain scales (EQUUS-FAP for foals C.A. 0,96, p<0,001 and HPF for foals C.A. 0,86, p<0,001). The EQUUS-FAP for foals was able to differentiate between the patient and control group (p<0,01), the HPF for foals was not able to differentiate between the two groups (p=0,063). Both pain scales were not able to differentiate between a limited number of foals from the patient group before and after administration of NSAIDs (EQUUS-FAP for foals p=0,416 and HPF for foals p=0,480). The cut-off value for differentiation between healthy and sick foals were determined at >2 for the EQUUS-FAP for foals and >1 for the HPF for foals. The sensitivity and specificity of the EQUUS-FAP for foals were good (90% and 86,36% respectively). The sensitivity of the HPF for foals was acceptable (70%), however the specificity of the HPF for foals was moderate (63,63%). The EQUUS-FAP for foals seems to be a promising tool for assessment of acute pain in foals aged from 14 days to 6 months. It is a repeatable and reliable facial expression-based pain scale and therefore this pain scale can be used for foals in acute pain. However, more research is necessary to further validate this pain scale.

Universiteit Utrecht

Table of contents

Introduction

The management of pain is a critical topic in equine veterinary medicine. By understanding the pain expression in patients and consequently develop a better system to manage the pain using analgesic drugs, the welfare of the patient can be improved. However, the understanding of pain in animals is complex and limited by the subjectivity of the response to pain. In addition animals cannot communicate pain verbally like humans can and horses specifically show their pain different in comparison to other animals for being flight animals. The instinct of the horse is to minimize the pain expression to prevent predation.[1] A definition of animal pain was described by Molony and Kent (1997)[2]: '*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.'* The behaviour of horses can be diverse for different types of injuries. The presence of pain in a horse can be detected by a change in the behaviour of the horse, however the expression of pain can have different dimensions. In example, severe abdominal disorders could be expressed by 'rolling', 'vocalisation', 'kicking at the abdomen', 'flank watching' and 'stretching', nevertheless it could also be expressed by severe depression with almost no signs on the outside. Furthermore, behavioural indicators for limb and foot pain and head and dental pain are described as completely different indicators in comparison to the abdominal pain indicators.[3] The understanding of the expression of pain in horses is complex and pain assessment methods were established to recognize and objectively score the severity of pain in adult horses. This could result in an improvement of the wellbeing of the patient in the equine veterinary clinic.[4,5]

The assessment of pain can be based on an interpretation of physiological parameters. The heart rate and respiratory rate are physiological parameters that are easily measured and can be a useful indicator of acute pain in the horse. The heart rate was defined by veterinarians in the UK as the primary parameter, alongside the assessment of the behaviour, to assess pain intensity in clinical cases.[6] Pritchett et al. found heart rates and plasma cortisol levels that were significantly higher in horses after postoperative exploratory celiotomy in comparison to the two control groups, consisting of one group that did not receive any treatment and one group that was anesthetized for a non-painful procedure.[7] However, the physiological parameters may be influenced by others factors, like excitement, cardiovascular and/or respiratory diseases, ambient temperature and dehydration[4] and a relation between the heart rate and pain was hard to prove.[8,9]

Behavioural assessment

Different types of behavioural assessment scales were constructed for the pain assessment of horses. Unidimensional scales were constructed to objectively assess the behavioural changes of horses in pain. First, the 'Visual Analog Scale' (VAS) is an easy to use pain assessment method. It consists of a 10 cm line which represents pain intensity from zero pain on one end of the line to maximum pain on the opposite end. An observer can put a mark on the line that indicates the intensity of pain he or she believes the horse is experiencing. A VAS score can be calculated with the length in millimetres from the zero end of the line to the mark on the line.[4] The VAS score was used in different studies to assess the pain of horses with acute colic or visceral pain with an inter-observer reliability from reasonable[10] to moderate[11]. Another example of an unidimensional scale is the ‘Numerical Rating Scale’ (NRS). The NRS has, in comparison to the VAS, pre-set numbers on a line. The observer can mark a number that indicates the pain a horse is experiencing.[4] Sutton et al.[12] used the NRS to assess the pain in horses with acute colic. The observer could score the pain of a horse from 1-6. In this study, a moderate inter-observer reliability was found for the NRS. The study of Pritchett et al.[7] describes nine behavioural parameters included in the NRS to assess pain in horses after exploratory celiotomy. An observer scores each behavioural parameter from 1-4 with a descriptive definition for each score. The NRS was able to differentiate between patients and control groups, consisting of one group that underwent anaesthesia for a non-painful procedure and one group without any treatment. A third unidimensional scale is the Simple Descriptive Scales (SDS). The SDS has prefixed classes with a number attached to it, for example, 0: none, 1: mild pain, 2: moderate pain and 3: severe pain. The Obel grading system is an example of a simple descriptive scale used for lameness in horses with laminitis.[13,14] The lameness of the horse is scored from 0 to 4, with 0: ‘no gait abnormalities’ and 4: ‘the horse experiences difficulty bearing weight at rest or is very reluctant to move’.[15]

Composite pain scale

A composite multifactorial pain scale is useful as a tool to assess the pain in horses with the combination of physiological parameters and behaviour of the patient. Bussières et al. (2008)[16] developed a composite pain scale for the assessment of pain in horses with acute orthopaedic pain. This composite pain scale describes behavioural changes, in two groups. In the first group, the reaction of the horse to the observer was assessed. The parameters in this group were ‘interactive behaviour’ and ‘response to palpation of painful area’. The second group assessed the behaviour of the horse itself. ‘Appearance’, ‘sweating’, ‘kicking at the abdomen’, ‘pawing on the floor’, ‘posture’, ‘head movement’ and ‘appetite’ were the parameters in this group. In each group a specific behavioural parameter can be scored from 0 to 3 with different criteria. The parameter ‘response to palpation of the painful area’ and ‘posture’ had a good to excellent specificity and sensitivity. Only one of the physiological parameters, the ‘Non-Invasive systematic arterial Blood Pressure’ (NIBP), had a good specificity and high sensitivity. However the heart and respiratory rate both had a moderate sensitivity and could not predict orthopaedic pain in horses. This composite pain scale was also used for the assessment of postoperative pain in patients after an emergency laparotomy. The pain scores in this study were significantly correlated with the clinical outcome of the patients and a high inter-observer reliability was obtained.[17] Additionally, the ‘Equine Utrecht University Scale for Composite Pain Assessment’ (EQUUS-COMPASS) is a composite pain scale based on the CPS by Bussières et al.[16] and modified by van Loon and van Dierendonck to assess acute visceral pain.[11] The behavioural parameters found to be most sensitive in this study, were ‘posture’, ‘sweating’, ‘reaction to observer’ and ‘reaction to palpation of the painful flank’. Later, the EQUUS-COMPASS was validated for the use in patients with acute colic.[18] The ‘Post Abdominal Surgery Pain Assesment Scale’ (PASPAS) is a composite pain scale constructed for the pain assessment of horses after abdominal surgery. In this composite pain scale, the behavioural parameters were divided into sub-categories that were characterised by specific descriptions of spontaneous, interactive and responsive behaviour. With a total maximum score of 30, a pain score below 7 was defined as low, between 7 and

14 was defined as moderate and a score above 14 was defined as a severe pain score. The inter-observer reliability of this pain scale was good. The physiological parameters did not correlate with the pain score, therefore the behavioural changes were more reliable to indicate acute abdominal pain.[19] The Unesp-Botucatu composite pain scale for mild to moderate acute pain after castration was constructed in 2015. The intra and inter-observer reliability in this study was variable for the parameters. This led to a refinement of the pain scale. Parameters with a good relevance, specificity and inter-item correlation were included in the refined composite pain scale. This composite pain scale was able to differentiate between the patients with acute mild to moderate pain and the healthy control group.[20]

Facial expression-based pain scales

In the human medicine, facial pain expressions have been studied to evaluate the pain of patients who cannot verbally express themselves, like paediatric patients or patients with verbal or cognitive disabilities.[21,22] Facial expressions of pain in neonates expressed when blood was collected, was already described in 1987.[23] In 1998, the pain faces in neonates were assessed by using the 'Neonatal Facial Coding System'(NFCS). The facial activity of the patients was increasing at the moment of an invasive event as well as the heartrate.[24] The 'Faces pain scale-Revised' (FPS-R) showed to be a useful method to score pain in children aged 4-5 years and older.[25] The 'Primal Face of Pain' (PFP) was evaluated by applying a computer-based methodology which measured facial movement in video's taken from neonates before and after a painful stimulus (heel-stick).[26] And the recent study of O'Neill et al. in 2019 shows a difference in facial expressions of pain in infants in different age groups from 2-6 months or six months and older. The difference in expression was suggested to be related to variances in regulatory capacity of infants 6 months or older, compared to the younger infants.[27]

In a study with experimentally induced pain, the changes in the facial expression of a horse were studied by inducing pain through applying a tourniquet on the antebrachium or an irritant on the skin.[28] This study indicates that horses have a facial expression that changes when they experience acute, experimentally induced pain. The 'Equine facial action coding system' (EquiFACS) was developed to look at the facial expression, but not only when the horse was in pain.[29] To use the facial expression in the veterinary practice, different facial pain scoring methods were developed. The 'Horse Grimace Scale' (HGS) was developed using stallions undergoing routine castration[30], later also described in horses with acute laminitis[31] and used to evaluate pain and the well-being of mares during Ovum-Pick up procedures.[32] The HGS consisted of six parameters to evaluate the facial expression of horses. The parameters used in this pain scale were 'stiffly backward 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'. Still images of videos of the face of the horses were evaluated by 5 observers. The HGS was found to be a reliable and effective method to assess pain in horses after routine castration.[30] The 'Equine Utrecht University Scale for Facial Assessment of Pain' (EQUUS-FAP) was developed scoring pain in horses with acute colic[11] and validated in comparison with the EQUUS-COMPASS.[18] After that, EQUUS-FAP was also described in horses with acute head-related pain[33] and in horses with acute and postoperative orthopaedic pain.[34] The EQUUS-FAP consisted of nine parameters, all scored from 0 to 2, with a total maximum pain score of 18. The nine parameters described in this pain scoring method were ‘Head’, ‘Eyelids’, ‘Focus’, ‘Nostrils’, ‘Corners mouth/lips’, ‘Muscle tone head’, ‘Flehming and/or yawning’, ‘Teeth grinding and/or moaning’ and ‘Ears’. Four observers assessed the facial pain of the horses in real life. The EQUUS-FAP had a high inter-observer reliability and can be a good pain assessment method to objectively score the facial pain expression of horses with acute pain.[11] Facial pain scoring methods are also used in other domestic and laboratory animals.[35-37] The ‘Rat Grimace Scale’ (RGS) used 4 parameters to assess the facial pain expression of laboratory rats.[37] Comparable to the facial pain scoring methods for horses described above, the RGS uses parameters like ‘Orbital tightening’ and ‘Ear changes’. The parameter ‘Nose/Cheek flattening’ concentrates on the shape of the nose and the cheeks, this could be slightly comparable to the parameters like ‘mouth strained and pronounced chin’ of the HGS. However, the last parameter of the RGS, the ‘Whisker changes’, is not seen in the methods for the facial expression of horses. The ‘Sheep Pain Facial Expression Scale’ (SPFES) used five parameters to assess the facial expression of sheep with foot rot. The parameters that are scored are similar to the parameters used in the HGS and the EQUUS-FAP. In sheep and pigs, grimace scales were developed for neonates or young animals to assess pain by the facial expression during management procedures like tail docking and castration.[38,39] The ‘Lamb Grimace Scale’ (LGS) consisted of five parameters. In comparison to the SPFES for adult sheep, one parameter is not similar, namely the parameter ‘Cheek flattening’ (bulging of the cheeks) in the LGS is not seen in the SPFES, were the cheeks are assessed on tightening of the masseter muscle.[38]

In comparison to humans and other domestic animals, foals were not yet included in the previous studies developing facial pain scores in horses.[18,30] The goals of the current study are to construct a facial expression-based pain scale for foals aged two weeks to six months old, using the EQUUS-FAP and Horse Pain Face (the Horse Pain Face is still under construction at the Utrecht University), to assess inter- and intra-observer reliability and to determine the differences in facial pain expression between healthy foals and foals with acute painful conditions, like colic, arthritis or respiratory infections and in foals with postoperative pain. The hypotheses are that the adjusted EQUUS-FAP and Horse Pain Face are both clinically applicable in foals with acute pain and both pain scales can differentiate between healthy foals and foals with acute pain.

Materials and methods

Animals

Ten sick foals between the age of 14 days and 6 months that have been admitted to an equine clinic in the Netherlands, Germany or Ireland were included in this study. The group existed of eight warmblood foals and two thoroughbred foals. The foals in the patient group were admitted to the clinics for different reasons, namely: traumatic wounds, colic, abdominal rupture and surgery (fractures, overbite correction or umbilical hernia). Foals in the control group (n=22) were filmed at several breeding stables in the Netherlands and in Ireland. The foals in the control group were all warmblood foals. All foals in the control group were healthy and free from acute diseases, based on the information of the owner or caretaker. Owners were informed about the study and gave their written consent for all foals included in this study.

Table 1
Data of the horses that were included in de study (n=32)

		Patients	Control
Number of foals		10	22
Sex	Mares	10	14
	Stallion	0	8
Age (days)	Mean	127,6	108,01
	Standard deviation	53,13	45,73

Video clips

Video clips of 30 seconds long were collected for this study. The video clips were taken from healthy foals and patients. The video clips were taken at different veterinary clinics in the Netherlands, Germany and Ireland. The video clips were taken with the mare and foal in a horsebox and the person with the camera outside the box. The focus of the camera is on the head of the foal. If the foals needed surgery, the foals were filmed before surgery and two hours after leaving the recovery box, if possible. When the foals received any pain medication, the foals were filmed before and after receiving their treatment if possible. The video clips were assessed by two trained veterinary students of Utrecht University and a specialist in equine anaesthesiology using the 'Equine Utrecht University Scale for Facial Assessment of Pain for foals' and the 'Horse Pain Face for foals'. The video clips were randomized and blinded, therefore the observers had no knowledge of the health status of the foals and the treatment protocols.

The 'Equine Utrecht University Scale for Facial Assessment of Pain' (EQUUS-FAP) for foals

The EQUUS-FAP for foals used in this study was based on the EQUUS-FAP for neonates, which was constructed based on the 'Equine Utrecht University Scale for Facial Assessment of Pain' for adult horses with acute pain created by van Loon and van Dierendonck.[11] The EQUUS-FAP for foals (table 2) was based on eleven parameters describing different features of facial expression. The parameters are scored from 0 to 2, with a total maximum of 22 points. The parameters 'yawning, 'smacking', 'teeth grinding' and 'moaning' could only be scored 0 or 2. The EQUUS-FAP for foals deviates from the EQUUS-FAP for adult horses in a couple of parameters. First, the parameters 'flehming and/or yawning' was changed in 'yawning' for the EQUUS-FAP for foals. Secondly, the parameter 'teeth grinding and/or moaning' was separated in two parameters. And finally, the parameter 'smacking' was added in the EQUUS-FAP for foals.

Table 2: EQUUS-FAP for foals

Data	Categories	Score
Head	Normal head movement Less movement/increased movement No movement/strongly increased movement	0 1 2
Eyelids	Opened, sclera can be seen in case of eye/head movement More opened eyes/tightening of eyelids Obviously more opened eyes/obvious tightening of eyelids	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 slightly Obviously lifted	0 1 2
Muscle tone head	No fasciculations Mild fasciculations Obvious fasciculations	0 1 2
Yawning	Not seen Seen	0 2
Smacking	Not seen Seen	0 2
Teeth grinding	Heard Not heard	0 2
Moaning	Heard Not 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		../22

The 'Horse Pain Face' scale for foals

The 'Horse Pain Face' (HPF) is under construction at the Utrecht University for a computer based pain scoring method. In this study, the HPF for neonates was used to assess the pain in foals. It consisted of five parameters of the facial expression of a horse and was developed on a picture based scale. The HPF for foals (table 3) used in this study deviates from the original HPF. The parameter 'visibility of the sclera' was removed from the pain scale in the HPF for foals. The five parameters used in the assessment of the group in this study were 'ears', 'orbital tightening', 'angulated upper eyelid', 'corners of the mouth/lips' and 'nostrils'. Each parameter was scored from 0 to 2 with a total maximum of 10 points.

Table 3: Horse Pain Face for foals

Ears	Both ears turned forwards At least one ear lateral position or further to backwards Both ears turned backwards	0 1 2
Orbital Tightening	Relaxed A bit tightening of the eyelids Obviously tightening of eyelids / eye closed	0 1 2
Angulated upper eyelid	Relaxed A bit more visible Obviously more visible	0 1 2
Corners mouth/lips	Relaxed Lifted a bit Obviously lifted / strained	0 1 2
Nostrils	Relaxed A bit more opened Obviously more opened (dilated mediolaterally)	0 1 2
Total		../10

Scoring

The video clips were observed and scored by three observers: observer 1 (specialist in equine anaesthesiology) and observer 2 and 3 (trained veterinary students from Utrecht University). Observer 1 scored the randomized and blinded video clips once. Observer 2 and 3 scored the video clips twice. Each video clip was shown twice during one scoring round and both scores were performed. All scores were collected in an excel sheet. The observers did not discuss their findings.

Data processing and statistical analysis

Inter-observer reliability was calculated using Intraclass Correlation Coefficient (ICC) for the total scores and each individual parameter. Cronbach's alpha was used to determine the correlation over all three observers. Scatterplots were used to display the correlations between the observers. Intra-observer reliability for observer 2 and 3 was calculated using the Intraclass Correlation Coefficient (ICC) and scatterplots were used to display the correlation between the first and second time of scoring. From this point, because of the excellent ICC between observer 2 and 3, the mean of the scores of observer 2 and 3 were used for further calculations in this study. Differences between the patient and control group were displayed in boxplots and analysed using the Mann-Whitney U test. The scores used for this test was the score of the patient where the peak of the pain was expected (in example before NSAID's or after surgery). Using the boxplots, cut-off values were determined to achieve a maximal difference between the patient and control group. These cut-off values were then used to calculate the sensitivity and specificity for the total scores of the EQUUS-FAP for foals and the HPF for foals. The sensitivity and specificity for the individual parameters of the scoring methods was calculated using the scores of 0 as a negative and >0 as positive. The difference between patients before and after receiving treatment with NSAIDs was displayed in boxplots and analysed using the Wilcoxon signed rank test. The programme that was used for the statistical analyses was SPSS. Statistical significance was accepted at $p<0.05$.

Results

Inter-observer reliability

Figure 1 shows the correlation between the pain scores from the three observers for the EQUUS-FAP for foals and the HPF for foals. The EQUUS-FAP for foals and the HPF for foals both showed a significant correlation between the observers (Cronbach's alpha of 0,90, $p<0,001$ for EQUUS-FAP for foals, Cronbach's alpha of 0,71, $p<0,001$ for HPF for foals).

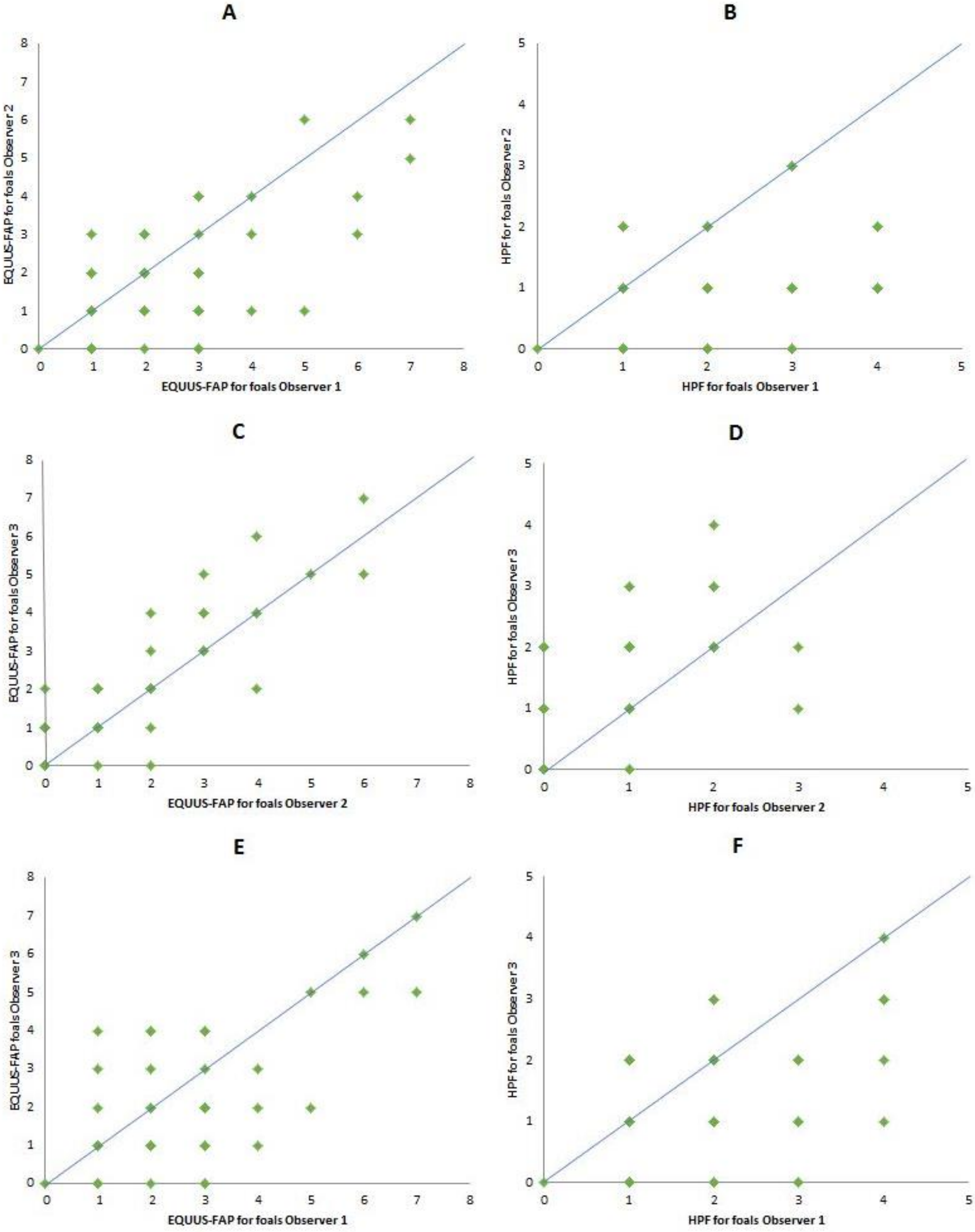

Figure 1: Scatterplots showing the correlation between the three different observers of the 'EQUUS-FAP for foals score and 'Horse Pain Face for foals' score of three observers (n= 48, blue line; y=x, ICC= intraclass correlation). (A) EQUUS-FAP for foals correlation between observer 1 and 2, ICC=0,70, (B) HPF for foals correlation between observer 1 and 2, ICC=0,45, (C) EQUUS-FAP for foals correlation between observer 2 and 3, ICC=0,86, (D) HPF for foals correlation between observer 2 and 3, ICC=0,54, (E) EQUUS-FAP for foals correlation between observer 1 and 3, ICC=0,69, (F) HPF for foals correlation between observer 1 and 3, ICC=0,40.

The ICC between the three observers for the different parameters and the total score of the EQUUS-FAP for foals is shown in table 4. The ICC between the three observers for the different parameters and the total score of the HPF for foals is shown in table 5.

Table 4: Intraclass correlation coefficient (ICC) between three observers of the EQUUS-FAP for foals score for eleven parameters and total score. Cronbach's Alpha (C.A) is showing the correlation between the scores of the three observers. P-value is showing the significance of the intraclass correlation coefficient. * Scores of parameter 'Muscle tone head' were not useable because two of the three observers never scored higher than 0. ** Scores of parameter 'Teeth grinding' was only useable for observer 2 and 3, because observer 1 did not score higher than 0 (n=48).

	Head	Eyelids	Focus	Nostrils	Corners mouth/ lips	Muscle tone head *	Yawnin g	Smacking	Teeth grinding **	Moaning	Ears	EQUUS -FAP for foals
ICC Obs. 1-2	-0,05	0,21	0,69	-0,03	0,14	-	1	0,43	-	1	0,84	0,70
ICC Obs. 2-3	0,76	0,39	0,61	0,18	0,41	-	1	0,71	-0,04	1	0,65	0,86
ICC Obs. 1-3	-0,07	0,18	0,64	0,37	0,56	-	1	0,54	-	1	0,64	0,69
C.A.	0,48	0,47	0,83	0,40	0,64	-	1	0,79	-	1	0,88	0,90
P-value	0,004	0,005	<0,001	0,19	<0,001	-	<0,001	<0,001	-	<0,001	<0,001	<0,001
Limits of agreemen t	0,16-0,69	-0,06–0,53	0,72-0,90	0.03-0.62	0,33-0,76	-	1-1	0,66-0,88	-	1-1	0,80-0,93	0,79-0,93

Table 5: Intraclass correlation coefficient between three observers of the scores of the HPF for foals for five parameters and total HPF for foals score. Cronbach's Alpha (C.A) is showing the correlation between the scores of the three observers. P-value is showing the significance of the intraclass correlation coefficient (n=48).

	Ears	Orbital tightening	Angulated Upper Eyelid	Corners mouth/lips	Nostrils	HPF for foals
ICC Observer 1-2	0,84	0,51	0,30	0,16	0,02	0,45
ICC Observer 2-3	0,64	0,57	0,50	0,41	0,15	0,54
ICC Observer 1-3	0,65	0,35	0,33	0,58	0,37	0,40
C.A.	0,88	0,71	0,60	0,65	0,41	0,71
P-value	<0,001	<0,001	<0,001	<0,001	0,016	<0,001
Limits of agreement	0,80-0,93	0,50-0,82	0,23-0,72	0,37-0,77	0,04-0,62	0,29-0,78

Intra-observer reliability

The correlation between the first and second time scoring with the EQUUS-FAP for foals and the HPF for foals combined for observer 2 and 3 is shown in figure 2. The scores of the EQUUS-FAP for foals and the HPF for foals both show a significant correlation between the first and second time scoring of the two observers. The EQUUS-FAP for foals has a C.A. of 0,96, the limits of agreement of 0,94-0,97 and p<0,001. The HPF for foals has a C.A. of 0,86, the limits of agreement of 0,79-0,91 and p<0,001.

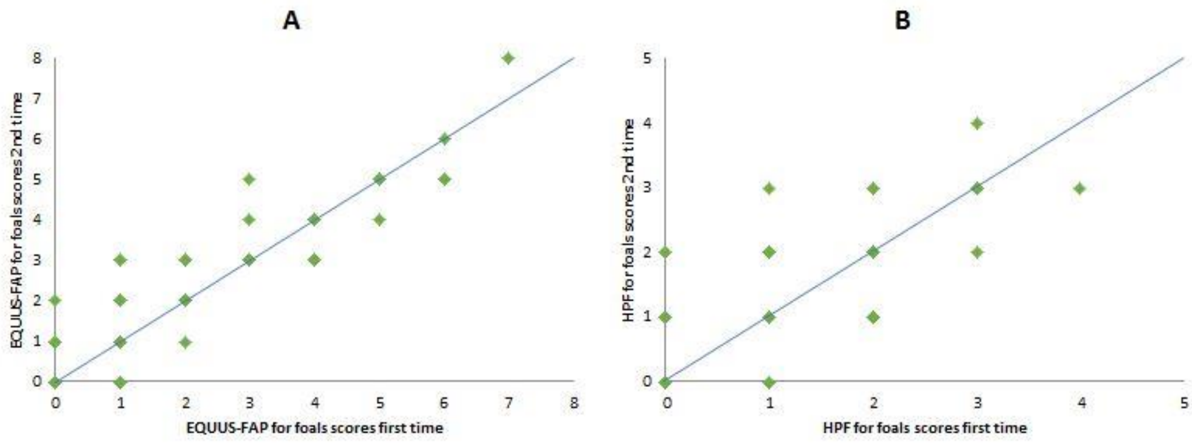

Figure 2: Scatterplots showing the intra-observer correlation between the scores of two observer scoring the first time and the second time with the EQUUS-FAP for foals and the HPF for foals (n=96, blue line; y=x, C.A.=Cronbach's alpha). (A) Correlation of the EQUUS-FAP for foals scores between the first and second time scoring of two observers, C.A.=0,96, p<0,001, (B) Correlation of the HPF for foals scores between the first and second time scoring of two observers, C.A.=0,86, p<0,001

Difference between the patient and control group

The difference between the patients and the control group is shown in figure 3. The scores of the EQUUS-FAP for foals show a significant difference between the two groups (p<0,01). The scores of the HPF for foals did not show a significant difference (p=0,063).

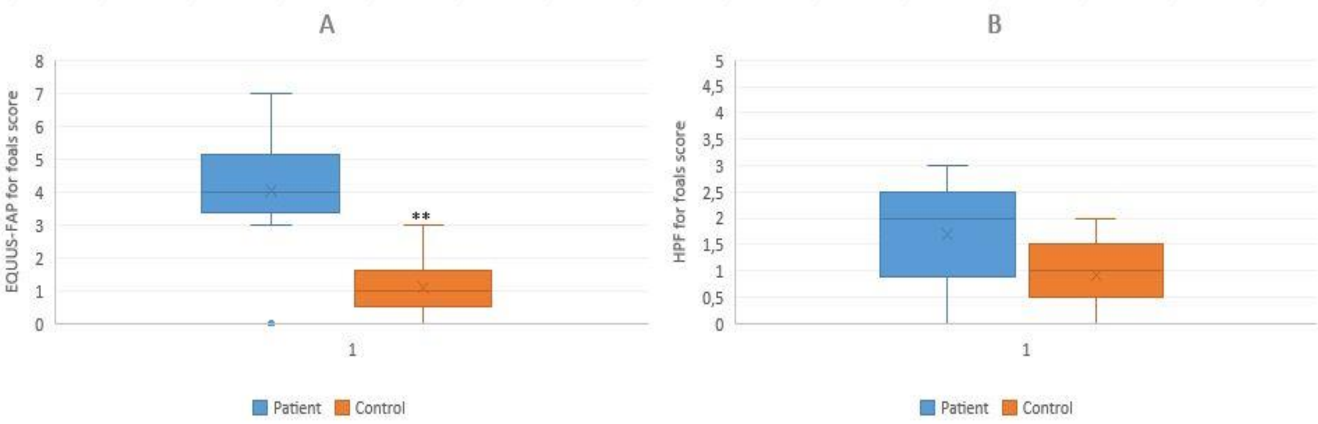

Figure 3: Boxplots showing the difference in scores of the patient and control group of the EQUUS-FAP for foals and the HPF for foals. (n=32, 10 patients, 22 controls), **=p<0,01. (A) EQUUS-FAP for foals. (B) HPF for foals, p= 0,063. The line in the box shows the median score. The boxes show the 25-75th percentiles and the ranges are indicated by the whiskers.

Difference between the scores before and after administration of NSAIDs

The scores of patients treated with NSAID's that were observed before and after the treatment are shown in figure 4. The mean scores of two observers did not show a significant difference before and after NSAID treatment for both the EQUUS-FAP for foals (p=0,42) and the HPF for foals (p=0,48).

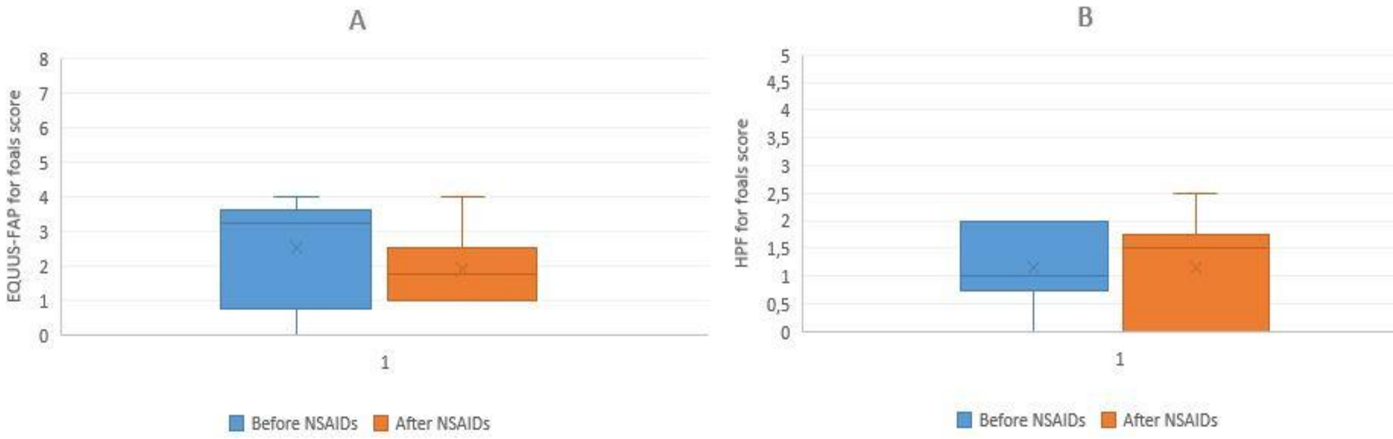

Figure 4: Boxplots of the scores of patients treated with NSAID's before and after receiving NSAID's (n=6). (A) EQUUS-FAP for foals, p=0,416. (B) HPF for foals, p=0,480. The line in the box shows the median score. The boxes show the 25-75th percentiles and the ranges are indicated by the whiskers.

Sensitivity and specificity of the scoring methods

The sensitivity and specificity are shown in table 6 (EQUUS-FAP for foals) and table 7 (HPF for foals) for individual parameters. The cut-off values used to calculate the sensitivity and specificity of the total scores were >2 for the EQUUS-FAP for foals and >1 for the HPF for foals.

Table 6: Sensitivity and specificity of the individual parameters of the EQUUS-FAP for foals in percentage and a sensitivity and specificity of the total EQUUS-FAP for foals score with a cut-off value of >2 (n=32, 10 patients, 22 controls).

	Sensitivity %	Specificity %
Head	30	100
Eyelids	50	90.91
Focus	40	100
Nostrils	70	22.73
Corners mouth/lips	40	90.91
Muscle tone head	0.0	100
Yawning	10	100
Smacking	60	77.27
Teeth grinding	0.0	100
Moaning	0.0	100
Ears	50	86.36
Total FAP Score	90	86.36

Table 7: Sensitivity and specificity of the individual parameters of the HPF for foals in percentage and a sensitivity and specificity of the total HPF for foals score with a cut-off value of >1 (n=32, 10 patients, 22 controls).

	Sensitivity %	Specificity %
Ears	50	86.64
Orbital tightening	50	90.91
Angulated upper eyelid	10	59.10
Corners mouth/lips	40	90.91
Nostrils	70	22.73
Total HPF score	70	63.63

Discussion

This study describes the evaluation of the 'Equine Utrecht University Scale for Facial Assessment of Pain for foals' and the 'Horse Pain Face for foals', both methods to objectively score the facial expression of pain in foals in the age of 14 days to 6 months. Both the inter- and intra-observer reliability were very good for the EQUUS-FAP for foals and were moderate to good for the HPF for foals. The EQUUS-FAP for foals was able to differentiate between the healthy foals and the patient group. Both the sensitivity and specificity of the total scores were excellent for the EQUUS-FAP for foals. The HPF for foals had a good sensitivity for the total score, however a lower specificity.

The EQUUS-FAP for foals shows a good inter-observer reliability, thus the behaviour of the foals in the video clips was interpreted mostly the same way by all three observers. The original EQUUS-FAP was constructed for adult horses with acute colic and scored a high inter-observer reliability (ICC=0,93)[11]. However the ICC of the original study was calculated with only two observers. Furthermore, the observing of the horses and scoring of the facial pain expression was done in real life in comparison to the current study with video clips. A difference between real life scoring and the scoring of video clips can be explained by the difficulty to score the video clips due to the short time (+/-30 seconds) of observation and the sometimes bad quality of the video clips. In real life, an observer can take its time to assess all parameters and if one parameter is not visible, the observer can wait for a better moment to score the parameter. This is not possible in a video clip of 30 seconds. In addition, the observer of the video clips does not know what the foal did just before the recording. The observer could see behaviour for an expression of pain, while it was actually normal behaviour. Nevertheless, an advantage of the scoring of video clips is that the three observers in this current study all scored the exact same 30 seconds, therefore they all saw the exact same behaviour. The 'Horse Grimace Scale' (HGS) was developed using images extracted from video recordings of horses after routine castration and identified a difference in scoring images due to quality of the images and the difference in coat colour of the horses.[30] The colour of the coat of a horse in combination with bad lighting or bad image quality, can make it difficult to assess all the parameters correctly on the face of a horse. The different quality of the images was also a limitation in the 'Mice Grimace Scale' development, were the observers could score better with a higher quality of the images.[36] The study of Gleerup et al.[28] describing the Equine Pain Face scored real life horses and video clips of horses. They described the effect of an observer on the facial expression. The facial expression was not suppressed by the horses in the presence of an observer, however the facial expression were less prominent when it tried to interact with an observer.

The intra-observer reliability of the EQUUS-FAP for foals was excellent, therefore the scoring of observer 2 and 3 was consistent for the first and second time scoring. The EQUUS-FAP for foals seems to be reproducible and reliable for different observers. Nevertheless the observers in this study were all trained before the scoring. The correlation between observer 2 and 3 was the highest and an explanation for this can be the training of these observers. Observer 2 and 3 had the exact same training, observer 1 was a specialist in equine anaesthesiology with more experience in this field. In addition, this cannot be compared with owners or veterinarians who could use this scale for facial pain scoring in the future. The EQUUS-FAP for foals should be used with some training in advance to recognize the right individual parameters. The inter-observer reliability of the individual parameters shows the difference between parameters that are 'easy' to score and are 'difficult' to score. The parameters 'Focus', 'Smacking', 'Moaning', 'Yawning' and 'Ears' have a high correlation between the three observers. These parameters may be easy to recognize, even if the observers did not have the same training in advance. Specifically 'Yawning' is a parameter that is easily seen, it is scored or it is not scored, therefore it gets a 0 or a 2 in this scoring method. The other parameters, 'Head, 'Eyelids', 'Nostrils' and 'Corners of mouth/lips' with a lower correlation between the three observers may be parameters that need extra attention in a training before scoring foals with the EQUUS-FAP. Each parameter should get a short video clip or image of a foal in a training module, presenting the score 0,1 or 2. Therefore the observer can estimate if their foal is in one of these groups. The parameters that need extra attention, can have more information and more video clips to see different foals with the different scores. This way, the observer can already see some foals in different stages of pain.

The specificity of the individual parameters of the EQUUS-FAP for foals is overall excellent. One parameter has a low specificity of 22,73%, namely the parameter 'Nostrils'. The specificity of this parameter is very low, but the sensitivity is good (70%). This parameter was both seen in the patient and control group and therefore might not be able to differentiate between the two groups. This was also seen in a group of adult horses with colic versus control group [11]. An explanation for these scores of sensitivity and specificity can be the cut-off value. In the current study, a cut-off value of >0 was used for the calculation of the specificity. If the cut-off value was higher, in example >1, the specificity of 'Nostrils' may score higher if patients score more 2's and control group foals score more 1's. Another explanation why this parameter is not able to differentiate between the patient and control group may be the previously described low inter-observer reliability of this parameter. If the parameter was easy to score by all observers and had a higher reliability, it could result in a higher specificity and sensitivity. Therefore, the parameter 'Nostrils' can still be valuable for the scoring of acute pain scoring the facial expression. The sensitivity of the individual parameters of the EQUUS-FAP for foals are not all high, but sensitivity of the total EQUUS-FAP for foals score was very high (90%) with a cut-off value of >2. The individual parameters are not all seen in the patient group, but they do contribute to the high sensitivity in the total score. In addition, the specificity of the total score is very high (86,36%), which makes the EQUUS-FAP for foals a good scoring method of the facial expression in foals.

The HPF for foals had a lower sensitivity and specificity for the total HPF score. The inter-observer reliability over all three observers was good for the HPF for foals (C.A. = 0,71). However the ICC between the individual observers did not show a good correlation. The intra-observer reliability was excellent, consequently it is reproducible by the same observer. The individual parameters of this pain scale score moderate ICC's and moderate C.A. with one excellent parameter, namely 'Ears'. The correlation of this parameter is the exact same one as for the EQUUS-FAP for foals, thus if it was scored for the EQUUS-FAP for foals it was also scored for the HPF for foals by all three observers. The HPF for foals has a wider limits of agreement in comparison to the limits of agreement of the total score of the EQUUS-FAP for foals. The study of E. Bos in 2020 (unpublished) scored neonatal foals with the same facial expression based pain scales that were used in the current study. In the study of E. Bos, a smaller limits of agreement was found for both the HPF for foals and the EQUUS-FAP for foals. The difference in these limits of agreement between the two studies could be the amount of video clips scored in the studies. In the current study were more video clips scored. This could result in more deviation between the scores.

A focus in both pain scoring methods in this current study is the eye region. Nevertheless, described differently in both methods. In the EQUUS-FAP for foals is the parameter ‘Eyelids’ used and described as: ‘more opened eyes/tightening of the eyelids’. In comparison, two different parameters in the HPF for foals are described for the eye region, namely ‘Orbital tightening’ and ‘Angulated upper eyelids’. Both opening and closing of the eyes or eyelids is seen in other studies and seems essential in a pain scale. In the study of Dalla Costa et al.[30], horses in pain after routine castration showed high scores for ‘orbital tightening’. The study of Gleerup et al.[28] described widening of the eyes and the visibility of the sclera in the equine pain face. In comparison, with the construction of the EquiFACS, even five separate parameters for the eyes were established: ‘Inner brow raiser’, ‘Eye closure’, ‘Blink’, ‘Half Blink’ and ‘Upper Lid Raiser’.[29] The EquiFACS focusses on the anatomy of the head and the muscles to make a facial expression and therefore has parameters for each different muscle. Both facial-expression based pain scales used in the current study include the opening and closing of the eyes, however in different parameters. The ICC and C.A. for the two separate parameters of the HPF for foals are higher and thus more reliable between observers in comparison to the single parameter of the EQUUF-FAP for foals. However, the sensitivity and specificity of ‘eyelids’ from the EQUUS-FAP for foals and the ‘Orbital tightening’ of the HPF for foals is the exact same with a very high specificity (90,91%). Thus, both these parameters are barely seen in the control group and seem to be valuable parameters to have in a facial-expression based pain scale. The parameter ‘Angulated upper eyelid’ of the HPF for foals has a very low sensitivity (10%) and a low specificity (59,10%), thus the ‘Angulated upper eyelid’ is seen frequently in the control group and is barely seen in the patient group. The low sensitivity and specificity could be a reason to exclude this parameter in the future, however the inter-observer reliability of this parameter was only 0.60 (Cronbach’s Alpha), that could indicate an improvement in scoring of the parameter after a better training of the observers. In addition, if this parameter is excluded, the opening of the eye is not represented in the HPF for foals, while this was described above as an essential parameter for a facial-expression based pain scale.

In this study, the foals that received NSAIDs were filmed and scored before and after receiving their treatment to see if both facial pain scoring scales could differentiate between the two moments. Both methods did not show a significant decrease in pain scores after NSAID treatment. However, the boxplot of the EQUUS-FAP for foals seems to show a higher score for the video clips of patients before receiving NSAID treatment. A limitation in this study was the group of suitable patients used for this calculation. The group only existed of 6 patients that received NSAIDs and where filmed before and after the treatment. If a bigger group of patients can be collected in the future, the EQUUS-FAP for foals may have a better outcome. Another limitation of the current study is the presence of bandages or catheters in the patients. The observers were not totally blinded for the condition of the foal in all cases, due to the necessary catheters or bandages. This could have influenced the scoring by the observers. However, the use of bandages or catheters is expected for foals in acute pain or after surgery. To avoid this limitation, it is necessary to avoid filming the bandages or catheters during the recording of the foal and focus on the head of the foal.

Additionally, a limitation of this study was the presence of the person who filmed the foals, both in the control group and patient group. The presence of the person who filmed the foals might influence the facial expressions of a foal. The human contact foals had in advance of this study can influence the reaction it is showing towards humans when being filmed. This can be explained by several reasons. At first the study of Diugan et al. in 2014[40] describes a different reaction to humans at different ages of unweaned foals. The foals aged 20 weeks were friendlier to humans in comparison to the foals at a younger age. The behaviour towards humans can become more friendly and interactive with age. Secondly, the foals that had more handling by humans can become less scared from humans in comparison to foals that did not experienced human contact. Therefore the last group could react scared when being filmed by an observer. The study of Ligout et al. in 2008[41] found forced contact with weaned foals for 14 days to improve the relationship between humans and the foals in comparison to the control group that did not have any human contact. However, the better reaction of the foals that did have forced human contact did not last in an unfamiliar environment. A third reason for the foals to react to a person who filmed the foal could be the relationship of the mare with humans. The foals can be influenced by the reaction of the mother to humans. The study of Henry et al. in 2005[42] describes a better reaction of foals towards humans if in the first days of the foals life, the mare has positive contact with humans. Foals of protective mares were more distant and less approachable by humans in comparison to the friendly mares and foals. These studies did not describe anything about the facial expression of the foals and in this current study, we do not know how much contact each foal had before filming. However it can be a reasonable assumption that the human who is filming the foal can have an influence on the reaction of the foals and the mares that are with the foals. In the study of Gleerup et al. in 2015[28] describing the Equine Pain Face, the horses that were in pain, induced by a noxious stimulus, came to seek for more contact with a familiar person, however this was not significant. In a following study, the influence of humans on the facial expression of foals can be eliminated by recording the foals with cameras. The cameras have to be set up in the box for a longer period, consequently the mare and foal are used to the presence of the cameras.

Previous studies tried to develop facial-expression based pain scales for young animals. The ‘Piglet Grimace Scale’ (PGS) was developed in 2017 and the ‘Lamb Grimace Scale’ (LGS) was developed in 2016. In the PGS, only 3 main features were used to score the pain of the young animals, namely ‘orbital tightening’, ‘ear position’ and ‘cheek tightening/nose bulging’.[39] The first two are comparable with the parameters ‘Eyelids’ and ‘Ears’ of the EQUUS-FAP for foals and the ‘orbital tightening’ and ‘ears’ of the HPF for foals. However the last parameter ‘cheek tightening/nose bulging’ could be compared to the parameter ‘corners mouth/lips’ of both pain scales used in this current study. The other features of the head are not discussed in this PGS. The LGS consisted of 5 parameters that were scored on lambs undergoing tail docking. Only two of the parameters showed a significant difference between the scoring of lambs before and after the tail docking, namely ‘orbital tightening’ and ‘mouth features’.[38] In contrast, in the current study are these parameters like ‘Eyelids’ ‘Corner mouth/lips’ and ‘orbital tightening’ not the most reliable and sensitive parameters.

A following study can be necessary to further construct and validate these pain scales. The limitations discussed above, like bandages/catheters or persons outside the box influencing the pain expression, should be prevented in the future. An opportunity in a following study can be to collect more video clips of more patients and healthy foals in the age group of 14 days to 6 months. This will result in a bigger patient and control group that can make the results more reliable, in particular the results of the effect of NSAIDs given to patients. If a bigger group of patient data is collected, the results could be significant. Another opportunity for a following study could be the effect of training of the observers on the scoring. If a useful training method is produced and the scoring can be compared before and after training, the effect of training could explain some differences between the parameters. In the end, the pain scales are developed to be used by veterinarians in the field and horse owners to detect pain better and earlier and therefore improve the welfare of the foal. The pain scales should be easy to use for these veterinarians and horse owners, and an effective training is an important part of the success of the pain scales.

Conclusion

This study describes the construction and evaluation of the 'Equine Utrecht University Scale for Facial Assessment of Pain for foals' and the 'Horse Pain Face for foals', both methods to objectively score the facial expression of pain in foals in the age of 14 days to 6 months. The EQUUS-FAP for foals has a good inter- and intra-observer reliability, it can differentiate between the patient and control group and has an excellent sensitivity and specificity. Therefore, it could be clinically applicable, reliable and repeatable in foals in the age of 14 days to 6 months. The HPF for foals had a poor to moderate inter-observer reliability, however a good intra-observer reliability. This pain scale was not able to differentiate between a patient and control group. The HPF for foals is not clinically applicable and reliable for this age group. Therefore, to score facial expression of pain in foals in the age of 14 days to 6 months, the EQUUS-FAP is the best facial expression-based pain scale to use. However, more research is necessary to validate this pain scale in the future before it can be used in clinical practice.

Acknowledgements

I would like to thank my supervisor dr. Thijs van Loon for his support and feedback during this research project, the veterinarians from the clinics in the Netherlands, Ireland and Germany who participated in this research project by collecting video clips of patients and control animals and Elisa Bos for scoring the video clips. I would also like to thank the owners of the patients for allowing me to use their foal in this study and also the owners of the control group foals: Equiception, stal van Hoof, and de Bemihoeve.

References

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

2. Molony V, Kent JE. Assessment of acute pain in farm animals using behavioral and physiological measurements. *J Anim Sci*. 1997;75(1):266-272.

3. Ashley FH, Waterman-Pearson AE, Whay HR. Behavioural assessment of pain in horses and donkeys: Application to clinical practice and future studies. *Equine Vet J*. 2005;37(6):565-575.

4. de Grauw JC, van Loon, J. P. A. M. Systematic pain assessment in horses. *The Veterinary Journal*. 2016;209:14-22.

5. Gleerup KB, Lindegaard C. Recognition and quantification of pain in horses: A tutorial review. *Equine Vet Educ*. 2016;28(1):47-57.

6. Price J, Marques JM, Welsh EM, Waran NK. Pilot epidemiological study of attitudes towards pain in horses. *Vet Rec*. 2002;151(19):570-575.

7. Pritchett LC, Ulibarri C, Roberts MC, Schneider RK, Sellon DC. Identification of potential physiological and behavioral indicators of postoperative pain in horses after exploratory celiotomy for colic. *Applied Animal Behaviour Science*. 2003;80(1):31-43.

8. Price J, Catriona S, Welsh EM, Waran NK. Preliminary evaluation of a behaviour-based system for assessment of post-operative pain in horses following arthroscopic surgery. *Veterinary Anaesthesia and Analgesia*. 2003;30(3):124-137.

9. Raekallio M, Taylor PM, Bennett RC. Preliminary investigations of pain and analgesia assessment in horses administered phenylbutazone or placebo after arthroscopic surgery. *Veterinary Surgery*. 1997;26(2):150-155.

10. Sutton GA, Dahan R, Turner D, Paltiel O. A behaviour-based pain scale for horses with acute colic: Scale construction. *The Veterinary Journal*. 2013;196(3):394-401.

11. van Loon, J. P. A. M., Van Dierendonck MC. Monitoring acute equine visceral pain with the equine utrecht university scale for composite pain assessment (EQUUS-COMPASS) and the equine utrecht university scale for facial assessment of pain (EQUUS-FAP): A scale-construction study. *The Veterinary Journal*. 2015;206(3):356-364.

12. Sutton GA, Paltiel O, Soffer M, Turner D. Validation of two behaviour-based pain scales for horses with acute colic. *The Veterinary Journal*. 2013;197(3):646-650.

13. Obel N. Studies on the histopathology of acute laminitis. *Studies on the histopathology of acute laminitis.* 1948.

14. Menzies-Gow NJ, Stevens KB, Sepulveda MF, Jarvis N, Marr CM. Repeatability and reproducibility of the obel grading system for equine laminitis. *Vet Rec*. 2010;167(2):52-55.

15. Owens JG, Kamerling SG, Stanton SR, Keowen ML. Effects of ketoprofen and phenylbutazone on chronic hoof pain and lameness in the horse. *Equine Vet J*. 1995;27(4):296-300.

16. Bussières G, Jacques C, Lainay O, et al. Development of a composite orthopaedic pain scale in horses. *Research in Veterinary Science*. 2008;85(2):294-306.

17. van Loon, J. P. A. M., Jonckheer-Sheehy VSM, Back W, René van Weeren P, Hellebrekers LJ. Monitoring equine visceral pain with a composite pain scale score and correlation with survival after emergency gastrointestinal surgery. *The Veterinary Journal*. 2014;200(1):109-115.

18. VanDierendonck MC, van Loon, J. P. A. M. Monitoring acute equine visceral pain with the equine utrecht university scale for composite pain assessment (EQUUS-COMPASS) and the equine utrecht university scale for facial assessment of pain (EQUUS-FAP): A validation study. *The Veterinary Journal*. 2016;216:175-177.

19. Graubner C, Gerber V, Doherr M, Spadavecchia C. Clinical application and reliability of a post abdominal surgery pain assessment scale (PASPAS) in horses. *The Veterinary Journal*. 2011;188(2):178-183.

20. Taffarel MO, Luna SPL, de Oliveira FA, et al. Refinement and partial validation of the UNESP-botucatu multidimensional composite pain scale for assessing postoperative pain in horses. *BMC veterinary research*. 2015;11(1):83.

21. Ahola Kohut S, Pillai Riddell R, Flora DB, Oster H. A longitudinal analysis of the development of infant facial expressions in response to acute pain: Immediate and regulatory expressions. *PAIN®*. 2012;153(12):2458-2465.

22. Prkachin KM, Solomon PE. The structure, reliability and validity of pain expression: Evidence from patients with shoulder pain. *PAIN*. 2008;139(2):267-274.

23. Grunau RVE, Craig KD. Pain expression in neonates: Facial action and cry. *Pain*. 1987;28(3):395-410.

24. Grunau RE, Oberlander T, Holsti L, Whitfield MF. Bedside application of the neonatal facial coding system in pain assessment of premature infants. *Pain*. 1998;76(3):277-286.

25. Hicks CL, von Baeyer CL, Spafford PA, van Korlaar I, Goodenough B. The faces pain scale – revised: Toward a common metric in pediatric pain measurement. *Pain*. 2001;93(2):173-183.

26. Schiavenato M, Byers JF, Scovanner P, et al. Neonatal pain facial expression: Evaluating the primal face of pain. *PAIN*. 2008;138(2):460-471.

27. O'Neill MC, Ahola Kohut S, Pillai Riddell R, Oster H. Age-related differences in the acute pain facial expression during infancy. *Eur J Pain*. 2019;0(0).

28. Gleerup KB, Forkman B, Lindegaard C, Andersen PH. An equine pain face. *Veterinary Anaesthesia and Analgesia*. 2015;42(1):103-114.

29. Wathan J, Burrows AM, Waller BM, McComb K. EquiFACS: The equine facial action coding system. *PLoS one*. 2015;10(8):e0131738.

30. Dalla Costa E, Minero M, Lebelt D, Stucke D, Canali E, Leach MC. Development of the horse grimace scale (HGS) as a pain assessment tool in horses undergoing routine castration. *PLoS one*. 2014;9(3):e92281.

31. Dalla Costa E, Stucke D, Dai F, Minero M, Leach M, Lebelt D. Using the horse grimace scale (HGS) to assess pain associated with acute laminitis in horses (equus caballus). *Animals*. 2016;6(8):47.

32. Diego R, Douet C, Reigner F, et al. Influence of transvaginal ultrasound-guided follicular punctures in the mare on heart rate, respiratory rate, facial expression changes, and salivary cortisol as pain scoring. *Theriogenology*. 2016;86(7):1757-1763.

33. van Loon, J. P. A. M., Van Dierendonck MC. Monitoring equine head-related pain with the equine utrecht university scale for facial assessment of pain (EQUUS-FAP). *The Veterinary Journal*. 2017;220:88-90.

34. van Loon, J. P. A. M., Van Dierendonck MC. Pain assessment in horses after orthopaedic surgery and with orthopaedic trauma. *The Veterinary Journal*. 2019;246:85-91.

35. McLennan KM, Rebelo CJ, Corke MJ, Holmes MA, Leach MC, Constantino-Casas F. Development of a facial expression scale using footrot and mastitis as models of pain in sheep. *Appl Anim Behav Sci*. 2016;176:19-26.

36. Langford DJ, Bailey AL, Chanda ML, et al. Coding of facial expressions of pain in the laboratory mouse. *Nature methods*. 2010;7(6):447.

37. Sotocinal SG, Sorge RE, Zaloum A, et al. The rat grimace scale: A partially automated method for quantifying pain in the laboratory rat via facial expressions. *Molecular pain*. 2011;7(1):55.

38. Guesgen MJ, Beausoleil NJ, Leach M, Minot EO, Stewart M, Stafford KJ. Coding and quantification of a facial expression for pain in lambs. *Behavioural Processes*. 2016;132:49-56.

39. Viscardi AV, Hunniford M, Lawlis P, Leach M, Turner PV. Development of a piglet grimace scale to evaluate piglet pain using facial expressions following castration and tail docking: A pilot study. *Frontiers in veterinary science*. 2017;4:51.

40. Diugan EA, Popescu S, Spinu M. Qualitative assessment of the young foals' human related behaviour. *Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca Veterinary Medicine*. 2014;71(2):517-518.

41. Ligout S, Bouissou M, Boivin X. Comparison of the effects of two different handling methods on the subsequent behaviour of anglo-arabian foals toward humans and handling. *Applied Animal Behaviour Science*. 2008;113(1):175-188.

42. Henry S, Hemery D, Richard M-, Hausberger M. Human–mare relationships and behaviour of foals toward humans. *Applied Animal Behaviour Science*. 2005;93(3):341-362.