Monitoring acute pain in neonatal foals with the Equine Utrecht University Scale for Facial Assessment of Pain for foals (EQUUS-FAP for foals) and the Horse Pain Face for foals (HPF for foals)

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Abstract

Animal pain and pain assessment have received increasing attention during the past years. But, objectively assessing pain in horses is difficult, because horses will avoid predation by not openly expressing their pain. Various different pain assessment systems have been described in horses, such as unidimensional pain scales, composite pain scales and facial expression-based pain scales. Although pain assessment in adult horses has been investigated, there is no literature describing pain assessment in neonatal foals. In this study, two facial-expression based pain scales, the Equine Utrecht University Scale for Facial Assessment of Pain for foals (EQUUS-FAP for foals) and the Horse Pain Face for foals (HPF for foals), are constructed and described in neonatal foals.
For this study design, 26 neonatal foals aged between 0 and 14 days were used (n=9 patients; n=17 controls). All the foals were filmed in the stable with the mare by an observer outside the box. The video clips were randomized, blinded and scored by means of the EQUUS-FAP for foals and the HPF for foals by three independent observers.
Both the EQUUS-FAP for foals and the HPF for foals scores demonstrated high inter-observer reliability (Cronbach's alpha = 0,97 for EQUUS-FAP for foals; Cronbach's alpha = 0,93 for HPF for foals) with limits of agreement of 0,97-0,99 for EQUUS-FAP for foals and 0,84-0,96 for HPF for foals (p<0,001). Both HPF for foals and EQUUS-FAP for foals scores showed a high intra-observer reliability (Cronbach's alpha = 0,98 for EQUUS-FAP for foals; Cronbach's alpha = 0,94 for HPF for foals) and limits of agreement of 0,97-0,99 for EQUUS-FAP for foals and 0,91-0,97 for HPF for foals (p<0,001). Patients showed significantly higher pain scores compared to control foals (p<0,01 for EQUUS-FAP for foals and p<0,01 for HPF for foals). Nonsteroidal anti-inflammatory drug (NSAID) treatment decreased both pain scores, but differences were not significant (p=0,14 for EQUUS-FAP for foals and p=0,14 for HPF for foals).
The EQUUS-FAP for foals and the HPF for foals proved to be useful for pain assessment in neonatal foals in accordance to the inter- and intra-observer reliability and differences between patient and control group. There were differences between scores before and after NSAID treatment, but they were not significant, possibly due to a small number of included animals (n=4). This study demonstrated that both pain scales are reliable for facial-expression based pain scoring of neonatal foals, of which the EQUUS-FAP for foals appeared to be more reliable, repeatable and clinically applicable. Further research in neonatal foals is necessary to investigate validity and practical applicability of both pain scales.

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Introduction

Animal pain

For a long time, people refused to believe that animals are capable of experiencing pain (Molony and Kent 1997). But nowadays animal pain has received increasing attention. Although animals will not be conscious of the term pain or suffering, animals can experience pain (Rietmann et al. 2004, Van Loon et al. 2014). In order to investigate and treat pain, it is important to define pain. The International Association for the Study of Pain (IASP) defined pain as an "*unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage*" (Merskey and Bogduk 1994). But for animal pain, there is a more appropriate definition: "*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; unnecessary pain occurs when the intensity or duration of the experience is inappropriate for the damage sustained or when the physiologic and behavioral responses to it are unsuccessful at alleviating it.*" (Muir 2010). Because pain is a subjective experience, it can be difficult to objectively assess (De Grauw and van Loon 2016). These difficulties in pain assessment are particularly complex in horses, because horses are prey animals and openly expressing pain is a sign of weakness which makes them more vulnerable for predators (Taylor et al. 2002, De Grauw and van Loon 2016).

Distinguishing pain from stress is another challenge in veterinary medicine. Pain can lead to stress, but stress does not always come with pain. Physical activity or physiological strain can also initiate a stress response (Rietmann et al. 2004). Muir (2010) defined stress as "*Responses to acute injury or environmental change that leads to an increase in metabolic rate, blood clotting, water retention and immune function; a 'fight' or 'flight' alarm reaction with autonomic activation*". Fear, injury, physiological strain or physical activity can cause the body's homeostasis to reestablish with a stress response by increasing the sympathetic activity and endogenous catecholamines. This results in an increase in heart and respiratory rate, and an increase in temperature and blood pressure causing a decrease in pain perception. Because pain can also cause these increases, it can be hard to distinguish from stress. Then, fear causes a 'fight-fright-flight' reaction (and a decrease in pain perception). Anxiety on the other hand, caused by a possible threat in the future, is characterized by the releasement of adrenocorticotropic hormones leading to an increase in pain perception (Wagner 2010).

Nociception

Knowledge of anatomy, physiology, pathologic processes and nociception is needed for objectively identifying pain in animals and neonates for adequate pain assessment, curative treatment and animal welfare (Rietmann et al. 2004, Muir 2010, Van Loon and van Dierendonck 2015).

Nociception is the neural process to detect (transduce) and process actual or potentially tissue-damaging stimuli. Neural processes covered by nociception are transduction, transmission, modulation, and perception. Nociception starts with transduction in the periphery at the nociceptors which convey mechanical, chemical, electrical and thermal stimuli to the central nervous system with electrical impulses. The nociceptive neurons express TRPs (transient receptor potential) on the surface, which immediately detect a noxious stimulus. Noxious stimuli are converted by nociceptors into an electrical nerve impulse, also called an action potential. Then, the action potential is transmitted to the spinal cord by unmyelinated or myelinated nerve fibers, resulting in the first trigger aversion and withdrawal from the painful stimulus (perception). Those nociceptive mechanisms prevent or minimize tissue injury by protecting and aiding in tissue healing and repairing. An acute inflammatory response occurs when noxious stimuli cause tissue injury. Chemical substances such as prostaglandins, bradykinin, histamine and leukotrienes will be released, which decreases normal nociceptive threshold and results in peripheral sensitization (increased sensitivity to noxious stimuli) or hyperalgesia (prolonged response to tissue-damaging stimuli). Peripheral sensitization can initiate central sensitization caused by chronic pain. Central sensitization is the increased sensitivity of central nociceptors to normal afferent input. It can last for days and is characterized by secondary hypersensitivity (pain outside the damaged area), hyperalgesia (increased response to noxious stimuli) and allodynia (stimulus that normally does not cause pain) (Muir 2010).

Pain related parameters

Over the years many studies have been performed to assess pain in horses. Equine practitioners have tried to objectify and simplify the diagnosis of pain in horses, but fully objective assessment of pain is currently impossible looking at the nature of pain (Taylor et al. 2002).
Many different pain scoring systems have been developed, but not all include the required criteria for valid pain scoring. According to Van Loon and van Dierendonck (2018), a valid pain scale should ideally produce reproducible scores, should be ease to use, practically applicable and it should be understandable for users. Furthermore, it should ideally produce instant results (Van Loon and van Dierendonck 2018).
One of the approaches to study pain is to formulate pain-related parameters and assess these. Parameters related to pain can be physiologic, endocrine and behavioral parameters. Measuring and quantifying physiological parameters such as heart and respiratory rate, may give an indication of the presence and severity of pain. But, to date, there is no clear established relation between pain and physiological parameters, because these parameters are not specific for the severity and presence of pain. The increase of those parameters can also be initiated by dehydration, stress, excitement and respiratory or heart diseases (Muir 2010, Wagner 2010, De Grauw and van Loon 2016).
Other parameters used for pain assessment can be endocrine parameters, such as catecholamines, endogenous cortisol and β-endorphins. These parameters are also correlated to stress, which makes it hard to distinguish between increases in endocrine parameters caused by stress, pain or a combination (Rietmann et al. 2004, De Grauw and van Loon 2016). On the other hand, endocrine parameters should be measured in the lab, which takes time and makes quick pain assessment in the field difficult (De Grauw and van Loon 2016).
Behavioral parameters are subjective parameters that could be seen as a result of pain or stress. Pain responses that could be seen after a painful event are: (1) avoiding recurrence of the painful experience, (2) withdrawal reflexes, (3) lying or standing still and (4) communication by posture, smell or vocalization (Molony and Kent 1997). Behavioral responses can also be caused by other sources of distress, such as unwillingness to riding or restlessness (Rietmann et al. 2004).
Pain assessment based on only behavior is difficult in clinics, because the behavior of the horse in another environment might be different from home and there is no reference of the behavior before the pain started (De Grauw and van Loon 2016). But, behavioral parameters can be used together with physiological parameters in a systematic pain assessment system (Gleerup et al. 2015).

Systematic pain assessment

Systematic pain assessment systems are constructed of behavioral parameters or a combination of both physiological and behavioral parameters. Systematic scales and scoring systems will help to improve the recognition of pain and should be reliable when used by different observers in different situations. Systematic pain scales are divided in three subgroups; unidimensional pain scales, composite pain scales and facial-expression based pain scales (De Grauw and van Loon 2016).

Unidimensional pain scales

Unidimensional pain scales are constructed of behavioral or physiological parameters. Examples of unidimensional pain scales are the Visual Analogue Scale (VAS), Numerical Rating Scale (NRS), Simple Descriptive Scale (SDS) and the Equine Acute Abdominal Pain Scale (EAAPS) (Wagner 2010, De Grauw and van Loon 2016, Sutton and Bar 2016).
The Visual Analogue Scale (VAS) is constructed of a 10 cm continuous scale representing pain intensity. The left part of the scale designates no pain and the right part the worst pain imaginable. Observers give a mark on the line that corresponds with the amount of pain the animal suffers from. The VAS score does not only appear to be suboptimal for horses depending on the amount of time observing a horse,

but the VAS is also very subjective, because observers score without structured and pre-defined classes. This is confirmed by a suboptimal inter-observer reliability (Sutton et al. 2013, De Grauw and van Loon 2016).
The numerical rating scale (NRS) is similar to the VAS and consists of a horizontal line with a score of 0 to 10 (0 representing no pain and 10 the worst pain). In contrast to the VAS, the NRS scale is not continuous. The observers give a mark closest to the amount of pain an animal is experiencing. The NRS appears to be more repeatable than the VAS, because it is less sensitive for small changes in pain, but scoring with the NRS is, like the VAS, not subjective (De Grauw and van Loon 2016). Van Loon and van Dierendonck (2015) compared the VAS with the EQUUS-COMPASS and the EQUUS-FAP and concluded a poor to moderate inter-observer reliability of the VAS in comparison to the EQUUS-COMPASS and the EQUUS-FAP.
A Simple Descriptive Scale (SDS) is an unidimensional pain scale that consists of pre-defined classes to which each class is assigned a number: 0, no pain; 1, mild pain; 2, moderate pain; 3, severe pain. These pain scales allow to run statistical tests on the data. An example of a SDS is the Equine Acute Abdominal Pain Scale (EAAPS) (De Grauw and van Loon 2016).
The Equine Acute Abdominal Pain Scale (EAAPS) is a behavior-based pain scale, developed for acute abdominal pain in horses. The EAAPS includes pre-defined classes like: kicking at the abdomen, pawing, weight shifting and rolling. Each class can be assigned by a 0, 1, 2 or 3. This pain scale showed in a follow-up study by Sutton and Bar (2016) a good reliability, validity and practical applicability (Sutton et al. 2013, Sutton and Bar 2016). This was also confirmed in a recent comparative study of Sutton et al (2019).

Composite pain scales

Composite pain scales combine multiple variables of behavioral parameters, physiological parameters or a combination of both (Lindegaard et al. 2010, Taffarel et al. 2015, De Grauw and van Loon 2016). During the time, various pain scales have been described for acute visceral or somatic pain in horses. An example of a composite multifactorial pain scale is the CPS, developed by Bussieres et al. (2008) for equine acute orthopedic pain. Although the CPS is developed for equine orthopedic pain, it has also shown to be useful for acute equine abdominal pain in the study of Van Loon et al. (2014). Another composite pain scale, which is developed for horses that had undergone laparotomy, is the 'post abdominal surgery pain assessment scale' (PASPAS) (Graubner et al. 2011). The PASPAS scale combines two categories, physiological and behavioral (interactional and observational) parameters (Graubner et al. 2011).
Then, the EQUUS-COMPASS (Equine Utrecht University Scale for Composite Pain Assessment), which is based on the CPS by Bussieres et al. (2008), is introduced by Van Loon and van Dierendonck (2015) for horses with acute abdominal pain. They adjusted the CPS to the EQUUS-COMPASS by adding more specific parameters for equine abdominal pain. During their study, it appeared that the EQUUS-COMPASS had a good validity, reproducibility and also showed to be useful for following horses over the time (Van Loon and van Dierendonck 2015, Van Dierendonck and van Loon 2016).

Facial-expression based pain scales

Facial-expression based pain scoring is an upcoming strategy for objective pain assessment in infants (Kohut et al. 2012) and recently in animals including lambs (Guesgen et al. 2016), horses (Dalla Costa et al. 2014), piglets (Viscardi et al. 2017), rabbits (Sotocina et al. 2011) and mice (Leach et al. 2012). Facial-expression based pain scoring systems are based on recording and quantifying facial expressions and have been proven reliable for pain assessment (Kohut et al. 2012, Van Dierendonck and van Loon 2016, Viscardi et al. 2017). This is confirmed by a study of Gleerup et al. (2015), which showed significantly higher pain scores after inducing experimental pain in adult horses. Facial expressions that changed after inducing pain with a tourniquet or topical capsaicin were; asymmetrical ears, dilated nostrils, angulated upper eyelids, tension of the lips and increased muscle tone of the head (Gleerup et al. 2015).
Compared to other methods of pain assessment in animals, facial expression-based pain scoring has a number of advantages. In first place, facial-expression based pain scoring take less time to carry out. In second place, untrained observers can be rapidly trained to use them. In third place, when scoring

pain, focusing on the face utilizes our potential tendency to look at faces. In fourth place, painful conditions from mild to severe, can be effectively assessed. Then, safety of the observers is another big advantage of facial-expression based pain scoring, observers can easily score from a safe distance (Dalla Costa et al. 2014). Researchers also studied the possibility for automated recognition of pain in adult humans with Facial Action Coding System (FACS), which describes changes in muscular movement of the facial musculature (Ashraf et al. 2009, Wathan et al. 2015). Wathan et al. (2015) described EquiFACS, a Facial Action Coding system for adult domestic horses, describing facial parameters with an objective coding system. They identified each facial movement with a code, an Action Unit (AU), which corresponds with studies in humans and other species. Results showed 17 Action Units (AUs), which was less than in humans (27 AUs), but a lot of similarities with human FACS were seen (Wathan et al. 2015). In various species, action coding systems have been identified to develop grimace scales (Dalla Costa et al. 2014). Recently, Viscardi et al. (2017) developed the Piglet Grimace Scale for piglets following tail docking and castration, and also evaluated the effectiveness of non-steroidal anti-inflammatory drugs (NSAIDs) after surgery. The authors concluded a significant difference between animals before surgery and up to 7 hours after the procedure (Viscardi et al. 2017). The Lamb Grimace Scale also showed a significant difference in pain scores between the control group and patients, and before and after tail docking (Guesgen et al. 2016).
In adult horses, Gleerup et al. (2015) described the Equine Pain Face, that is used after inducing experimental pain in horses. Another grimace scale for adult horses, is the Horse Grimace Scale (HGS), which has been described after surgical castrations and in horses with acute laminitis (Dalla Costa et al. 2014).
Recently, Van Loon and van Dierendonck (2015) developed the Equine University Utrecht Scale for Facial Assessment of Pain (EQUUS-FAP), which is constructed for facial-expression based pain scoring in adult horses with visceral pain, to obtain a more valid, clinically applicable and a reliable scoring system. The EQUUS-FAP also proved to be very useful and valid for horses with postoperative and acute head-related pain such as fractures and equine recurrent uveitis (Van Loon and van Dierendonck 2017), and in horses with acute or postoperative orthopedic pain (Van Loon and van Dierendonck 2019).
Although there is a lot of ongoing research about pain scoring in adult horses and other species, to date, there is no literature describing (facial-expression based) pain scoring in neonatal foals.

Aim of study

This clinical study aims to study pain in neonatal foals aged 0-14 days in a clinical situation in comparison to a control group of healthy, pain free foals by means of the Equine Utrecht University Scale for Facial Assessment of Pain for foals (EQUUS-FAP for foals) and the Horse Pain Face for foals (based on the Horse Pain Face, which is under construction at the Utrecht University, unpublished data). The hypotheses of this study are (1) that the EQUUS-FAP for foals and HPF for foals make it possible to differentiate between patients and controls, where the control group has a lower facial-expression based pain score than patients admitted to the referral clinic, (2) that both pain scales have good inter- and intra-observer reliability (3) that with both pain scales, a significant decrease in pain scores will be found after NSAID treatment.

Materials and methods

Animals

For this study design, two research groups (Table 1) of neonatal foals with an age up to fourteen days were formed. The first research group included nine foals that had been admitted to several equine referral clinics. The patients suffered from acute pain coming with colic, bladder rupture, gastric ulcers, laminitis or post-operative pain. The other group was a control group and contained seventeen healthy, pain free foals filmed at different stables in The Netherlands.
Some of the patients (6) were filmed twice: before and after administration of NSAIDs or pre- and post-OK. The total number of foals was 26 and the total number of video clips was 32.
The foals were from different kind of breeds, such as Thoroughbred, Haflinger, Friesian horse and Warmblood foals. No selection by gender took place. The owners of the foals provided written informed consent by signing a letter that explained the purpose of the study. The observers were not part of the patient care and did not know any of the foals that were admitted to the referral clinics. Table 1 shows details on numbers, gender and age of the foals that were enrolled in this study.

Table 1
Data of foals that were included in the study design (n=26).

		Patient	Control
Number of foals		9	17
Breed	Warmblood	5	11
	Thoroughbred	3	1
	Haflinger	0	5
	Friesian Horse	1	0
Sex	Mare	4	10
	Stallion	5	7
Age	1-7 days	2	16
	7-14 days	7	1

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

The EQUUS-FAP was constructed by Van Loon and van Dierendonck (2015) for facial-expression based pain scoring of adult horses with acute visceral pain, acute or postoperative head-related pain (Van Loon and van Dierendonck 2017) and recently in horses with orthopedic trauma or postoperative orthopedic pain (Van Loon and van Dierendonck 2019).
After a testing round, we decided to adjust the EQUUS-FAP for adult horses to the EQUUS-FAP for foals (Table 2). Adjustments made to the EQUUS-FAP were; addition of the parameter smacking and removal of the parameter flehming. The parameter smacking was added because it appeared that lots of foals (controls and patients) were smacking during the video clip, therefore it was interesting to determine whether smacking was related to pain or not. The parameter flehming was removed because it appeared that, during the video clips, only healthy, pain free foals were flehming. Then, the paired parameters yawning/smacking and teeth grinding/moaning were divided into single parameters. In addition, sclera, which was part of the parameter eyelids, was also removed, because the sclera appeared to be visible in healthy, pain free foals.
The EQUUS-FAP for foals includes the following eleven parameters; head movement, eyelids, focus on the environment, nostrils, corners of the mouth, muscle tone of the head, yawning, smacking, teeth

grinding, moaning and the position of the ears. Each parameter can be scored from 0 to 2, resulting in a maximal score of 22. A few weeks after the adjustments, the video clips were scored the second time with the EQUUS-FAP for foals so the observers would not recognize the foals in the video clips.

Table 2
Score sheet of the Equine Utrecht University Scale for Facial Pain Assessment for foals (EQUUS-FAP)

Data	Categories	Score
Head	Normal head movement	0
	Less movement/increased movement	1
	No movement/strongly increased movement	2
Eyelids	Opened, sclera can be seen in case of eye/head movement	0
	More opened eyes/tightening of eyelids	1
	Obviously more opened eyes/obvious tightening of eyelids	2
Focus	Focussed on environment	0
	Less focussed on environment	1
	Not focussed on environment	2
Nostrils	Relaxed	0
	A bit more opened	1
	Obviously more opened, nostril flaring and possibly audible breathing	2
Corners mouth/lips	Relaxed	0
	Lifted slightly	1
	Obviously lifted	2
Muscle tone head	No fasciculations	0
	Mild fasciculations	1
	Obvious fasciculations	2
Yawning	Not seen	0
	Seen	2
Smacking	Not seen	0
	Seen	2
Teeth grinding	Not heard	0
	Heard	2
Moaning	Not heard	0
	Heard	2
Ears	Position: orientation towards sound/clear response with both ears or ear closest to source	0
	Delayed/reduced response to sounds	1
	Position: backwards/no response to sounds	2
Total		../22

The Horse Pain Face for foals (HPF for foals)

The Horse Pain Face is a facial-expression based pain scale for adult horses that is under construction at the Utrecht University. This pain scale is tested for automated pain scoring of photos instead of video clips. Data of the HPF has not been published yet.
After a testing round, it also appeared that the Horse Pain Face needed a small adjustment to become the Horse Pain Face for foals (Table 3). The parameter 'sclera' was removed from the score sheet, because the sclera appeared to be visible in healthy, pain free foals and it was difficult to score on video clips instead of photos. Parameters scored by the Horse Pain Face for foals are; position of the ears, orbital tightening, angulated upper eyelid, corners of the mouth and nostrils. All parameters can be assigned a score of 0-2, resulting in a total maximal score of 10. In this study, the HPF for foals is used to compare the results with the EQUUS-FAP for foals.
A few weeks after the adjustment, the video clips were scored the second time.

Table 3
Score sheet of the Horse Pain Face for foals (HPF)

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

Experimental design

All the foals were filmed in the stable with the mare by an observer outside the box. All the video clips lasted at least thirty seconds and the head of the foal had to be clearly visible. The video clips were randomized, blinded and doubled by a specialist in Equine Anesthesia, who also scored the video clips (observer 1) once a few weeks after randomizing, blinding and doubling. The video clips were scored twice (randomized, blinded) by two veterinary medicine master students (observer 2 and 3) to assess inter and intra-observer reliability. The observers were allowed to watch each fragment twice, because it was impossible to score all 16 parameters at once in thirty seconds. During the testing round, it appeared that one video clip was not appropriate for facial-expression based pain scoring, because the head of the foal was not clearly visible. So, this video clip was excluded from our study.
The observers scored the foals based on their facial expressions with the Equine Utrecht University Scale for Facial Pain Assessment for foals (EQUUS-FAP for foals) and the Horse Pain Face for foals (HPF for foals). The observers did not discuss their findings.

Data processing and statistical analysis

To assess inter- and intra-observer reliability, intraclass correlation analysis was used. The first time scoring of observer 2 and 3 (n=64) was compared to the second time scoring of observer 2 and 3 to determine the intra-observer reliability. Because intra-observer reliability was very high, pain scores of both master student observers were averaged and these average pain scores were used for further analysis.
Differences in patient and control foals were determined with the Mann-Whitney U test. To obtain maximal differentiation between patient and control foals, cut-off values were determined for both EQUUS-FAP for foals and HPF for foals.
Internal specificity and sensitivity were determined for the total EQUUS-FAP for foals and HPF for foals score with the set cut-off values. Sensitivity and specificity of each individual parameter was determined. The Wilcoxon signed rank test was used to assess differences before and after NSAID treatment (n=4). Statistical analyses were performed with SPSS (version 20). Statistical significance was accepted at $p<0,05$.

Results

Inter-observer reliability

The correlation between the pain scores of the EQUUS-FAP for foals and the HPF for foals of three independent observers is shown in figure 1. The EQUUS-FAP for foals and the HPF both showed a significant correlation (p=<0,001 for both EQUUS-FAP for foals and HPF for foals) and a Cronbach's alpha of 0,97 for the EQUUS-FAP for foals and 0,93 for the HPF for foals. Limits of agreement for the EQUUS-FAP for foals was 0,97-0,99 and 0,84-0,96 for HPF for foals. The Intraclass Correlation Coefficient ICC of the three observers is shown in table 4.

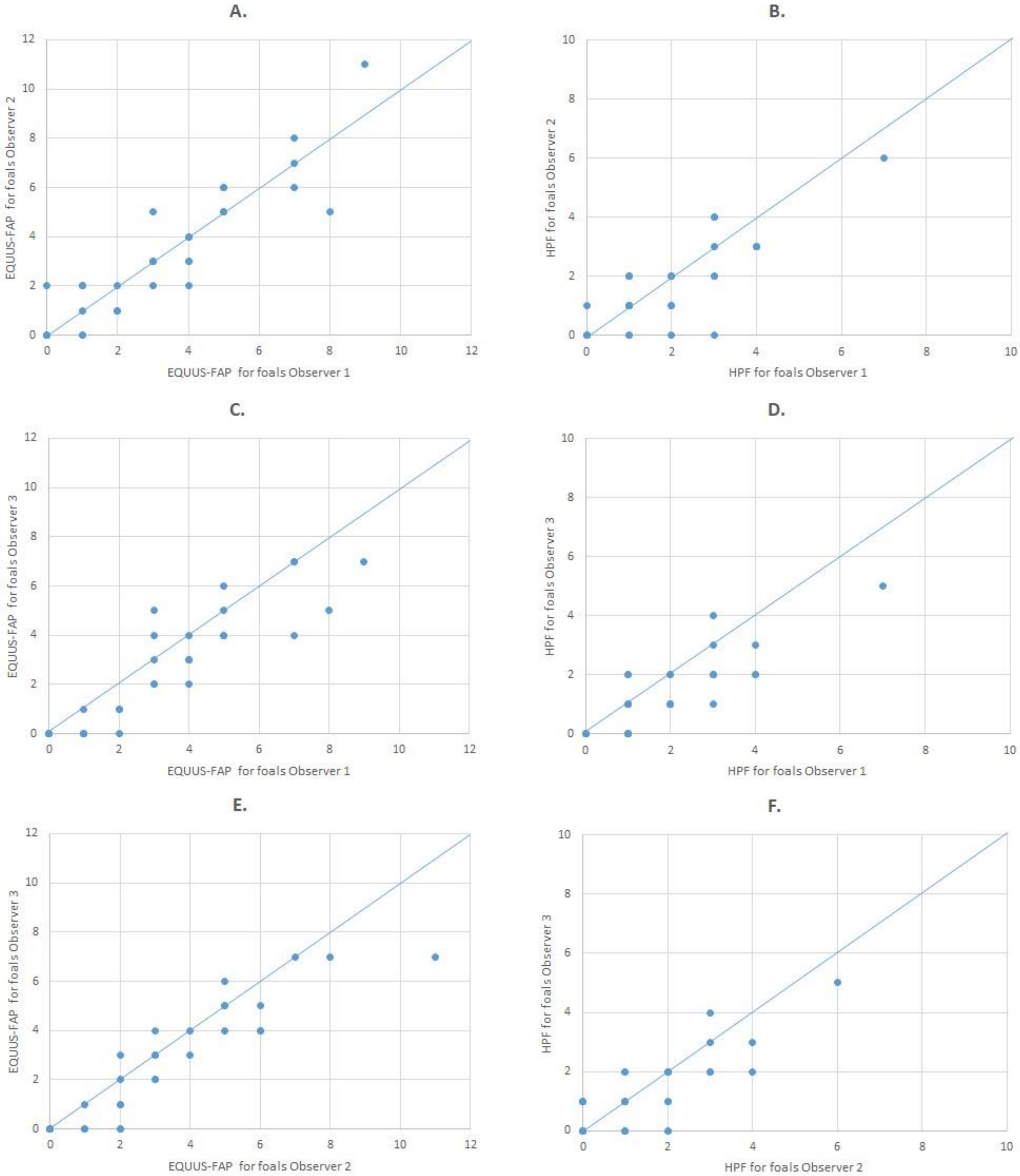

Figure 1*. Scatterplots describing the inter-observer correlation of the Equine University Utrecht Scale for Facial Assessment of Pain for foals (EQUUS-FAP for foals) and the Horse Pain Face for foals (HPF for foals) of three different, independent observers (n=32, blue line; y=x, ICC=intraclass correlation) (A) EQUUS-FAP for foals; correlation between observer 1 and 2, ICC=0,91(B) HPF for foals; correlation between observer 1 and 2, ICC=0,81 (C) EQUUS-FAP for foals; correlation between observer 1 and 3, ICC=0,84 (D) HPF for foals; correlation between observer 1 and 3, ICC=0,91 (E) EQUUS-FAP for foals; correlation between observer 2 and 3, ICC=0,92 (F) HPF for foals; correlation between observer 2 and 3, ICC=0,79*

Table 4

Intraclass correlation coefficient (ICC) between different observers scored with the HPF for foals (Horse Pain Face for foals) and the EQUUS-FAP for foals (Equine Utrecht University Scale for Facial Assessment of Pain for foals). Cronbach's Alpha (C.A.) describing the correlation between the scores of the three observers. P-value (p<0,001) showing the significance of the intraclass correlation coefficient. Limits of agreement describes the range that includes 95% of the scores.

	HORSE PAIN FACE FOR FOALS	EQUINE UTRECHT UNIVERSITY FOR FACIAL ASSESSMENT OF PAIN FOR FOALS
ICC OBSERVER 1-2	0,81	0,91
ICC OBSERVER 2-3	0,79	0,92
ICC OBSERVER 1-3	0,84	0,91
ICC C.A.	0,93	0,97
ICC P-VALUE	<0,001	<0,001
ICC LIMITS OF AGREEMENT	0,84-0,96	0,97-0,99

Intra-observer reliability

Figure 2 shows the correlation between the first and the second time the videos were scored by observer 2 and 3 (n=64). Both the HPF for foals and EQUUS-FAP for foals scores showed a significant correlation (p<0,001) with a Cronbach's alpha of 0,98 for the EQUUS-FAP for foals and a Cronbach's alpha of 0,94 for the HPF for foals. The limits of agreement showed a narrow range for both the EQUUS-FAP for foals (0,97-0,99) and the HPF for foals (0,91-0,97).

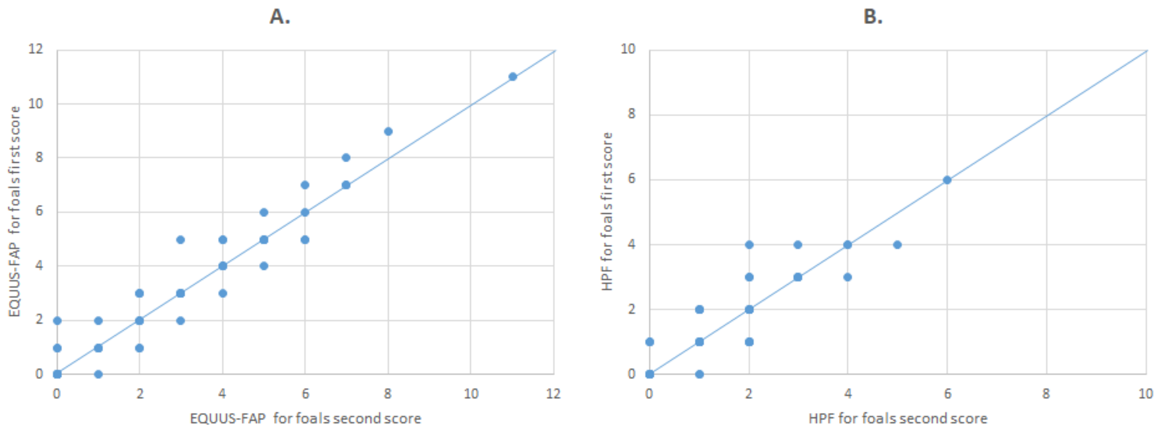

Figure 2. *Scatterplots describing the intra-observer correlation between the first and second time scoring with the Equine University Utrecht Scale for Facial Assessment of Pain for foals (EQUUS-FAP for foals) and Horse Pain Face for foals (HPF for foals) (n=64, blue line; y=x, C.A.=Cronbach's alpha)(A) Correlation of the EQUUS-FAP for foals between the first score and the second score, , C.A.=0,98, p=<0,001 (B) Correlation of the HPF for foals between the first and second score, C.A.=0,94, p=<0,001*

Differences between the patient and control group

Figure 3 shows the significant differences between the patient and control group for the EQUUS-FAP for foals (p<0,01) and the HPF for foals (p<0,01).

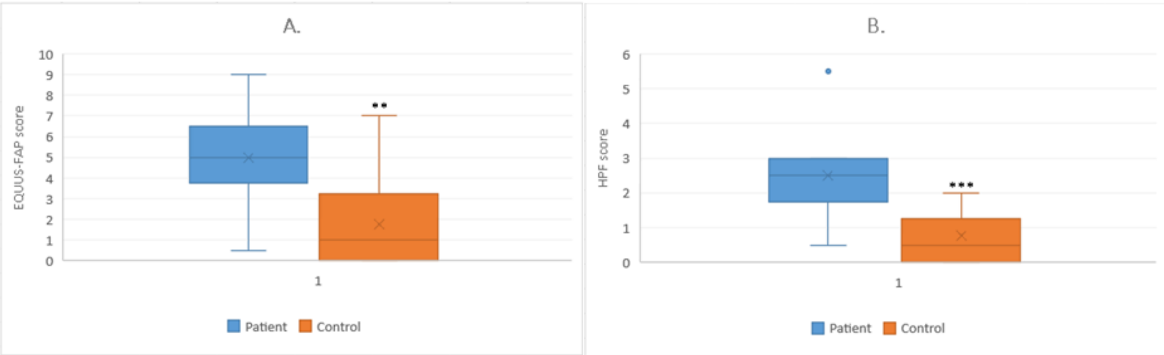

Figure 3. *Boxplots showing the differences between HPF for foals (Horse Pain Face for foals) and EQUUS-FAP for foals (Equine Utrecht University Scale for Facial Assessment of Pain for foals) scores of the patient and control group n=26 (9 patients, 17 control), **=p<0,01 ***=p<0,001 (A) EQUUS-FAP for foals, p=0,003 (B) HPF for foals, p=0,001. The median score is indicated by the line in the boxes, the first and third quartiles is indicated by the boxes and the ranges are indicated by the whiskers.*

Scores before and after NSAID treatment

The scores before and after administration of NSAIDs to the patients did not show significant differences for the EQUUS-FAP for foals (p=0,14) and the HPF foals (p=0,14) (Figure 4). However, the boxplots show that there is a difference between scores before and after administration of NSAIDs.

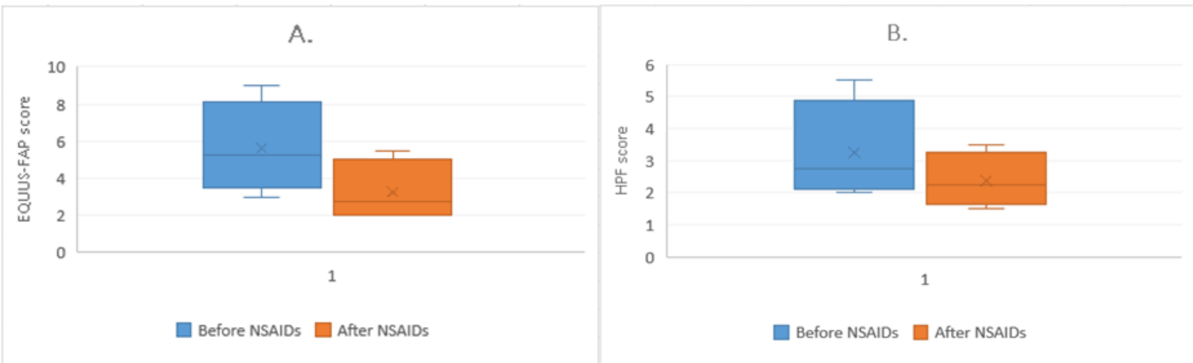

Figure 4. *Boxplots of EQUUS-FAP for foals (Equine Utrecht University Scale for Facial Assessment of Pain for foals) and HPF for foals (Horse Pain Face for foals) scores before and after NSAID treatment,(n=4 patients)(A) EQUUS-FAP for foals, p=0.14 (B) HPF for foals, p=0.14. The median score is indicated by the line in the boxes, the first and third quartiles is indicated by the boxes and the ranges are indicated by the whiskers.*

Table 5.
Sensitivity and specificity of the individual EQUUS-FAP for foals (Equine Utrecht University Scale for Facial Assessment of Pain for foals) parameters and the total EQUUS-FAP for foals score.

	Sensitivity	**Specificity**
Head	44.40%	100%
Eyelids	33.30%	88.20%
Focus	55.50%	88.20%
Nostrils	66.60%	58.80%
Corners of the mouth	66.60%	94.12%
Muscle tone head	44.40%	94.12%
Yawning	22.20%	88.20%
Smacking	55.50%	52.90%
Teeth grinding	0%	94.12%
Moaning	0%	100%
Ears	66.60%	88.20%
Total EQUUS-FAP for foals	**77.78%**	**76.47%**

Table 6.
Sensitivity and specificity of the individual HPF for foals (Horse Pain Face for foals) parameters and the total HPF for foals score.

	Sensitivity	**Specificity**
Ears	66.60%	82.30%
Orbital tightening	33.30%	88.20%
Angulated upper eyelid	33.30%	76.50%
Corners of the mouth	66.60%	94.10%
Nostrils	55.50%	52.90%
Total HPF for foals	**88.89%**	**76.47%**

Sensitivity and specificity of the individual parameters

Table 5 and 6 show the sensitivity and specificity of the individual EQUUS-FAP for foals (Table 5) and the HPF for foals (Table 6) parameters. None of the foals included in the patient group scored the parameters moaning and teeth grinding, although teeth grinding was scored in the control group.

Cut-off values of total EQUUS-FAP for foals and HPF for foals scores

For differentiation between patients and controls, cut-off values were determined. Cut-off values for EQUUS-FAP for foals were determined as >3 and for HPF for foals >1. Cut-off values were used to determine sensitivity and specificity of total EQUUS-FAP for foals and HPF for foals scores shown in table 5 and 6.

Discussion

This study demonstrates the construction and validation of the Equine Utrecht University Scale for Facial Assessment of Pain for foals (EQUUS-FAP for foals) and the Horse Pain Face for foals (HPF for foals) for facial-expression based pain assessment in neonatal foals. To assess pain with both pain scales, foals were stabled in their box with the mare. Patients that were admitted with acute painful disorders or that needed surgery in the referral clinic scored significantly higher than control foals. Foals that underwent surgery had relatively low pain scores at admission, but pain scores increased after surgery. After NSAID treatment, pain scores decreased or stayed the same, but the changes were not significant. This is in contrast to the study of Van Loon and van Dierendonck (2019), where pain scores significantly decreased after NSAID treatment. This is possibly due to a small research group of 4 foals in this study versus 43 horses in the study of Van Loon and van Dierendonck (2019). Scores given to patients in this study run up to 11/22 for the EQUUS-FAP for foals and 6/10 for the HPF for foals. Van Loon and van Dierendonck (2019) considered a total EQUUS-FAP score of 3-5 as mild pain, 5-8 as moderate pain and >8 as severe pain.
Table 7 shows the categories applied to the total EQUUS-FAP for foals scores of the foals included in this study. Only one post-surgery patient (before NSAID treatment) was scored with severe pain. The 2 foals that were scored with moderate pain were a post-surgery (before treatment with NSAID) patient and a patient that suffered from gastric ulcers. The categorization of the total EQUUS-FAP for foals pain scores should be further investigated in a new dataset.
Because data describing the HPF (for foals) results has not been published yet, there was no literature describing the categorization of the total pain scores. In this study, scores of 2-4 were considered as mild pain, 4-6 as moderate pain and >6 as severe pain (Table 8). The foal that was scored with severe pain by the EQUUS-FAP for foals (post-surgery patient before NSAID treatment), was scored with moderate pain by the HPF for foals. The difference in outcome might be due to the number of parameters scored (11 for EQUUS-FAP for foals and 5 for HPF for foals) and the maximal total score (22 for EQUUS-FAP for foals and 10 for HPF for foals). Besides that, the Horse Pain Face for foals is constructed for facial-expression based pain scoring of photographs instead of video clips. In a new dataset, the categorization of total HPF for foals pain sores should be further investigated.

***Table 7**. Total EQUUS-FAP for foals (Equine Utrecht University Scale for Facial Assessment of Pain for foals) pain scores divided in 4 groups (according to Van Loon and van Dierendonck 2019)*

	Patient	Control
<3	1	12
3-5	5	4
5-8	2	1
>8	1	0
Total	9	17

***Table 8**. Total HPF for foals (Horse Pain Face for foals) pain scores divided in 4 groups*

	Patient	Control
<2	2	15
2-4	6	2
4-6	1	0
>6	0	0
Total	9	17

Inter-observer reliability was assessed by means of intra class correlation analysis and showed a significant, strong correlation between the three independent observers and a high Cronbach's alpha for both pain scales. The EQUUS-FAP for foals also showed a strong and significant correlation in the study of orthopedic pain after surgery or trauma in adult horses of Van Loon and van Dierendonck (2019). The intra class correlation between the three observers and the Cronbach's alpha of the HPF for foals were lower but still significant in accordance to the intra class correlation of the EQUUS-FAP for foals. This difference could be due to less parameters scoring with the HPF for foals. The limits of agreement describes the range that includes 95% of the scores. Limits of agreement showed a narrow range for both the EQUUS-FAP for foals and the HPF for foals (0,97-0,99 for the EQUUS-FAP for foals and 0,84-0,96 for the HPF for foals).
Like the inter-observer reliability, the intra-observer reliability was assessed by means of intra class correlation analysis. For intra-observer reliability, the pain scores of two independent observers were

combined. For both the EQUUS-FAP for foals and the HPF for foals, significant and strong correlations were found. Limits of agreement for intra-class correlation also showed a narrow range for both the EQUUS-FAP for foals (0,97-0,99) and the HPF for foals (0,91-0,97). Because the intra-observer reliability was excellent for both the EQUUS-FAP for foals and the Horse Pain Face for foals, we were able to compare trained observers with each other and to reliably monitor patients during admission at the clinic. The observers in this study were trained to score the foals based on their facial-expressions. It is suspected that untrained observers (for example horse owners) could experience difficulties with scoring neonatal foals. But, after proper training, the EQUUS-FAP for foals and the Horse Pain Face for foals could potentially be used by horse owners and non-veterinarians.

To determine whether a parameter is specific for pain in neonatal foals or just part of normal facial expression of a healthy foal, the sensitivity and specificity were determined for all individual parameters for both the EQUUS-FAP for foals and the HPF for foals. EQUUS-FAP for foals parameters that scored >50% (for both sensitivity and specificity) were assumed as specific for pain in neonatal foals. Those parameters were; focus, corners of the mouth, nostrils, smacking and position of the ears. The EQUUS-FAP for foals parameters that appeared to be not specific for pain were; head, eyelids, muscle tone head, yawning, teeth grinding and moaning. Although those parameters were not specific in this study, we decided to keep them on the score sheet, considering future research with a bigger patient group.
In this study, none of the foals moaned. Moaning could be seen in very sick, painful intensive care patients (this category of patients was not present in our study). From the patient group, none of the foals scored the parameter teeth grinding, which is in contrast to some of the control foals. Considering future research with a bigger dataset, both parameters remain on the score sheet.

Cut-off value of the EQUUS-FAP for foals in this study is determined on >3 for patients and ≤3 for controls. This cut-off value corresponds to a previous study of Van Loon and van Dierendonck (2017) about monitoring equine head-related pain. The total EQUUS-FAP for foals score of patients and controls showed a moderate to good sensitivity of 77,8% and specificity of 76,5%. This is also similar to the results of Van Loon and van Dierendonck (2015) and Van Loon and van Dierendonck (2017), where both the sensitivity and specificity is determined between 80,0% and 90,0% in horses with head-related pain or acute visceral pain. Sensitivity and specificity should be determined in follow-up studies with a new dataset.

For the HPF for foals, the individual parameters ears, corners of the mouth and nostrils were most specific for facial-expression based pain scoring (>50% for both specificity and sensitivity) of the patient group, although the parameter nostrils was also scored in about 50% of the control foals. 60% of the patient group did not score positive for angulated upper eyelid and orbital tightening. This might be because the patient group did not include severe cases of illness with accompanying severe pain. In particular orbital tightening could be seen in severe cases of illness. Cut-off value for the HPF for foals was determined on >1 for patients and ≤1 for controls. The HPF for foals also scored high sensitivity (88,9%) and moderate specificity (76,5%) of the total score for patients and controls.

In contrast to the results of both the EQUUS-FAP for foals and the HPF for foals, the Rat Grimace Scale, which has been constructed for automated facial-expression based pain scoring of photographs in rats, all Action Units (AUs) were equally scored (AUs scored were; orbital tightening, nose/cheek flattening, ear changes and whisker change). The RGS appeared to be reliable and accurate, although it should be mentioned that they only scored 10 rats. The pain face of the rat also appeared to be almost equal to that of the mouse (Sotocinal et al. 2011). A study that also scored stills of videos is the study of Viscardi et al (2017). They scored piglets after tail docking and surgical castration with the Piglet Grimace Scale (PGS, 3 AUs; ear position, cheek tightening/nose bulge and orbital tightening) and looked if the pain grimacing correlated with the behavior of the piglets. All piglets displayed behavioral changes and there was a significant correlation between PGS scores and the behavior of the piglets. Then, the study of Guesgen et al. (2016) demonstrated the changes in facial expressions of 7 lambs before and after tail docking with the Lamb Grimace Scale (LGS, 5 AUs: orbital tightening, nose features, mouth features, cheek flattening and ear posture). They also scored the lambs on stills of video's (no stills available in their article). In lambs, ear posture was the most prominent parameter scored and, to a lesser extent, nose changes and orbital tightening. All those grimace scales share some similarities, but the differences between species in grimace scales and the number of Action Units may be due to different musculature, facial composition and differences in size and nature (prey or predator) of the animals.

Looking at the studies of Viscardi et al. (2011), Sotocinal et al. (2011) and Guesgen et al. (2016), the Horse Pain Face for foals comes closest to the grimace scales they constructed. The Horse Pain Face is also originally constructed for (automated) facial-expression based pain assessment of photographs instead of video clips. The Horse Pain Face for foals appeared in this study to be less suitable for pain scoring of video clips than the EQUUS-FAP for foals. Further research to test the Horse Pain Face and the Horse Pain Face for foals on photographs is necessary to validate both pain scales. But, looking at the results of the RGS, the PGS and the LGS the results might be promising.

Tabel 9. *The left column shows a control (healthy, pain free) animal and the right column shows a patient that is suffering from acute pain (Sotocinal et al. 2011, Viscardi et al. 2011)*

Control	Patient
	
	
	

Table 9 shows the differences between a pain free, healthy animal (control) and a patient (foal, piglet and rat). Although there are some differences between species, the animals in the left column have some things in common. None of the animals shows a tightening of the orbita, the ears are forward/in neutral position and the cheek and mouth are relaxed. This is in contrast to the animals in the right column, where the orbita is tightened and the ears are turned backwards in all animals. There are also some differences between the 3 patients. In response to cheek tightening, piglets that are in pain show a bulge of skin on the snout (Viscardi et al. 2017). Rats, on the other hand, show a flattening of the cheek and nose (Sotocinal et al. 2011), where foals obviously open their nostrils and lift the corners of their mouth when they are in pain. And when rats are in pain, they also change the position of the whiskers to a forward position (Sotocinal et al. 2011).

A limitation of this study was that data collection of the neonatal foals started and ended in the early foal season (February), which resulted in a small patient group. If the patient group would have included more foals, then the statistics of before and after NSAID treatment might have been significant. Another limitation was that both observer 2 and 3 knew some of the control foals, which also might have influenced the scoring. Besides that, some patients had an externally visible clinical condition like a catheter or bandages, which might have influenced the scoring in accordance to foals without a visible condition.

However, the EQUUS-FAP for foals and the HPF for foals need further validation in the future. A possibility to further validate both pain scales, is to determine differences in pain scores between different kind of breeds (for instance; Warmblood foals vs Coldblood foals). Van Loon and van Dierendonck investigated this difference between Warmblood and Coldblood horses in EQUUS-FAP and EQUUS-COMPASS scores in 2015 and also recently in 2019 (Van Loon and van Dierendonck 2015, Van Loon and van Dierendonck 2019). Both studies assessed no differences in pain scores between breeds. Van Loon and van Dierendonck (2017) also compared horses in another study with acute head-related trauma to horses with head-related post-operative pain. In the future, to validate the EQUUS-FAP for foals, comparing post-operative pain with other sources of acute pain is a possibility.

Conclusion

The aim of this study was to study pain in neonatal foals aged 0-14 days in a clinical situation in comparison to a control group of healthy, pain free foals by means of the Equine Utrecht University Scale for Facial Assessment of Pain for foals (EQUUS-FAP for foals) and the Horse Pain Face for foals (HPF for foals).
The hypotheses were to differentiate between patients and control foals, to apply good inter- and intra-observer reliability and to accomplish a significant difference in facial-expression based pain scores before and after treatment with non-steroidal anti-inflammatory drugs (NSAIDs). Both the HPF for foals and the EQUUS-FAP for foals accomplished a high inter- and intra-observer reliability for the assessment of pain in neonatal foals. In addition, both scales showed a significant difference between the control and patient group. This supports the first two hypotheses of this study. Differences in pain scores before and after NSAID treatment were not significant and should be tested with a bigger patient group. Thus, the third hypothesis should be rejected.
Previous scale validation studies demonstrate that the EQUUS-FAP can be used for repeatable and reliable pain assessment in adult horses. This study demonstrates that the EQUUS-FAP for foals is reliable for pain assessment in neonatal foals. The HPF for foals also demonstrated to be reliable for facial assessment of pain in neonatal foals, but resulted in lower inter- and intra-observer reliability. So, in this study, The EQUUS-FAP for foals appeared to be the most promising pain scale for facial-expression based pain scoring in neonatal foals, although further research is necessary to validate this pain scale.

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