

## **Introduction to Pain Mechanisms and Facial Expressions**

### **Article 1**

#### **Reference**

Viñuela-Fernández, I., Jones, E., Welsh, E. M., & Fleetwood-Walker, S. M. (2007). Pain mechanisms and their implication for the management of pain in farm and companion animals. *The Veterinary Journal*, 174(2), 227–239. <https://doi.org/10.1016/j.tvjl.2007.02.002>

#### **Summary**

The objectives of Viñuela-Fernández et al. (2007) were to consolidate research findings regarding neurophysiology of pain in animals. Their goal was to define the mechanism behind pain sensation/perception so that future studies could build on the information and develop new pain assessment techniques for farm animals. While most pain assessment tools in animals are centered around indirectly quantifying pain through changes in behaviour, this review was conducted to link pain to its direct physiological causes. The article reviewed works published between 1960-2006 on pain mechanisms of vertebrate animals, with a focus on how the information applies to farm (horse, cattle, pig) and companion (cats, dogs) animals. The major finding of the study was that the pathway behind sensing/perceiving noxious (i.e., painful) stimuli was the same regardless of its source (mechanical, thermal, or chemical). To relay information from the environment to the brain, the nervous system was found to depend on two specific types of fibers: thin-myelinated A $\delta$  fibers and unmyelinated C fiber. As the presence of painful stimuli is detected, the excitatory neurotransmitter glutamate is involved in helping the

electrical signal from the fibers reach the brain. Following activation of the neural pathway, researchers found that an immune response was also triggered to help with pain, mainly in the form of inflammation. The findings of this review are particularly significant as they suggest high conservation of the neuronal pain pathway in vertebrates. This means that studies conducted on commonly used test animals (such as mice) can be applied to animals that are more difficult to experiment on, such as horses. With an understanding of the pain mechanism, Viñuela-Fernández et al. (2007) concluded their review by suggesting that more research should be done to link specific injuries/diseases to their neurophysiological roots so more targeted treatment options can be designed.

### **Contribution**

I chose to include this article in my review as it provides a clear basis for the pain mechanism in vertebrates (including horses). Understanding the nervous and endocrine basis of pain is critical for developing a foundation for future research to stand on. With this review consolidating previous works and identifying a conserved mechanism for the pain pathway in vertebrates, future research can focus on where each species diverges and the effects that can have on the animal's experience of pain. Particularly, this research can be used to better understand the pain response observed in horses.

**Number of times this article has been cited: 170**

## Article 2

### Reference

Wathan, J., Burrows, A. M., Waller, B. M., & McComb, K. (2015). EquiFACS: The equine facial action coding system. *PLOS ONE*, *10*(8), e0131738.

<https://doi.org/10.1371/journal.pone.0131738>

### Summary

The goal of this study was to understand the physiology of a horse's head to develop an objective assessment tool that allows for understanding of their facial expressions. While previous works in the field focused on facial expressions made in response to specific events (such as the animal being in pain), Wathan et al (2015) created the Equine Facial Action Coding System (EquiFACS) to be able to describe all facial expressions made by horses regardless of their trigger. To develop EquiFACS, researchers first dissected a horse head to identify all muscles and ligaments present. Following identification of musculature, 15 hours of horse footage was observed to determine all the facial expressions naturally made by horses. By doing so, researchers were able to link horse facial expressions to their underlying physiology. Wathan et al. found that muscles of the eye and mouth (orbicularis oculi and levator nasolabialis muscles, respectively) were involved in making most facial expressions, especially those conveying pain and stress. To test the validity of EquiFACS as an objective assessment tool, four observers without previous experience with the system were asked to use it to identify facial expressions made by horses in a series of 22 video clips. The system demonstrated high inter-observer reliability with a Wexler's ratio of 0.86. The findings of this study are significant as not only do

they provide a reliable mechanism for detecting facial expressions of horses, but also because the most prominent facial expressions made by the horses were congruent with those found by past research. Findings of this study raise questions as to whether specific emotions/ types of pain are associated with contraction of specific facial muscles, or if only the general state of horses can be communicated by their facial expressions.

### **Contribution**

I chose this article for my review as it developed a novel technique for mapping facial expressions in horses. This study advanced work in its field by moving away from qualitative descriptions of facial expressions in horses and creating a mechanism to link external behaviours with the animal's underlying physiology. Findings of this study are congruent with previous works as they outline consistent contractions of eye/lip muscles in response to pain. Future research could work to identify whether specific muscle movements are associated with different emotional/physiological states in horses to better allow handlers to assess their animals' needs.

**Number of times this article has been cited: 146**

## Article 3

### Reference

Wathan, J., Proops, L., Grounds, K., & McComb, K. (2016). Horses discriminate between facial expressions of conspecifics. *Scientific Reports*, 6(1), 38322. <https://doi.org/10.1038/srep38322>

### Summary

The objective of this article was to determine whether horses are capable of distinguishing between different facial expressions made by other horses. Although previous research has established that horses make facial expressions to reflect their emotional/physiological states, no study has looked at whether horses can understand facial expressions made by their conspecifics (members of their species). The authors conducted two experiments to determine how aware horses were of facial expressions based on their interaction with 2 photographs of unfamiliar horses. One of the photographs depicted a horse with a positive facial expression (relaxed mouth/eyes, upward pointing ears), while the other showed a horse with a negative/aggressive facial expression (tense mouth/eyes, ears pointing backwards). In Study 1 (n=48), researchers looked to determine whether horses showed a preference towards either the positive or negative photo. In Study 2 (n=33), researchers observed how the horses' facial expressions changed in response to viewing each photograph. All observations were made using video-recordings of the animals. Results of Study 1 found that all the horses approached the photograph showing a positive facial expression first. Horses were also seen to spend more time with the positive photo (mean=24.48s) than the negative one (mean=1.29s). Results of Study 2 demonstrated that when looking at the negative photo, horses spent more time displaying

aggressive behaviours such as stiffening of the ears (mean=5.4s) compared to positive photo (mean=3.8s). These findings are not only significant because they suggest that horses understand the meaning behind different facial expressions, but also because they demonstrate facial expressions being used as a form of non-verbal communication among equines. A question raised by this article is whether horses can only distinguish between generally positive and negative facial expressions, or if they can identify specific emotions in their conspecifics as well.

### **Contribution**

I chose to include this article in my review as it provided further evidence of facial expressions being used as a form of non-verbal communication in horses. This article advanced knowledge in the field by demonstrating that horses are aware of/can understand the meaning of different facial expressions in their conspecifics. These results are congruent with past research as they demonstrate horses using facial expressions to communicate their emotions. Future research could aim to better understand how much horses can communicate with each other through their facial expressions.

**Number of times this article has been cited: 51**

## **Facial Expressions of Horses and Implications of Pain**

### **Article 4**

#### **Reference**

Gleerup, K. B., Forkman, B., Lindegaard, C., & Andersen, P. H. (2015). An equine pain face. *Veterinary Anaesthesia and Analgesia*, 42(1), 103–114. <https://doi.org/10.1111/vaa.12212>

#### **Summary**

The goal of this article was to identify and develop an ethogram of facial expressions horses made when experiencing pain. While previous works have explored equine pain face, they have not accounted for the influence of stress (from anaesthesia, medication, or being surrounded by unfamiliar humans) on facial expressions horses made during those studies. Thus, the goal of Gleerup et al. (2015) was to design an experiment that controlled for the effects of stress and specifically tested horses as they experienced acute (i.e., sudden) pain. To minimize stress caused by exposure to new people, researchers groomed and fed 6 healthy horses chosen for the study for 10 days before experiments began, thereby establishing a positive rapport with them. Following the 10-day familiarization period, pain was elicited in each horse by wrapping a tourniquet tightly around their forelimbs, and then applying a capsaicin cream on their hindlegs to create a burning sensation. Horse behaviour was monitored using video footage of the animals before and after stimulating pain. The main facial expressions researchers observed in horses experiencing pain were stiffening of the ears, squinting of the eyes, and clenching of the mouth. The facial expressions listed in this article were consistent with those described by previous

studies conducted on equine pain face. The most significant finding of this study was that the horses sought out their handlers when in pain. This finding is particularly noteworthy as horses have historically been known to distance themselves when in pain due to their nature as prey animals who try to conceal any signs of weakness from potential predators. With the discovery that in familiar environments horses move towards people they're comfortable with, this research raises questions regarding the comfort handlers can potentially bring their horses when they are in distress.

### **Contributions**

This study was included in my review as it provides a baseline for equine facial expressions of pain. This article advanced work in its field by focusing specifically on horses' response to noxious stimuli without the presence of stress. While facial indicators of pain found in this study are congruent with previous works, the discovery that horses seek comfort from familiar handlers when they're suffering is a novel finding not noted in literature before. Future studies could aim to better understand how horses perceive their handlers and how handlers can provide adequate support for horses in pain.

**Number of times this article has been cited: 208**



## Article 5

### Reference

Hintze, S., Smith, S., Patt, A., Bachmann, I., & Würbel, H. (2016). Are eyes a mirror of the soul? What eye wrinkles reveal about a horse's emotional state. *PLOS ONE*, *11*(10), e0164017.  
<https://doi.org/10.1371/journal.pone.0164017>

### Summary

The objective of this article was to determine whether changes in muscle contractions around the eyes could reflect the emotional state of horses. This study diverged from previous research that often focused on whether facial expressions could be used to detect pain and instead sought to discover if they can indicate horses' emotional states. Hintze et al. (2016) specifically looked at how contractions of the inner eyebrow muscles (levator anguli oculi and corrugator supercilli muscle) could be linked to expression of emotions by horses. The experiment exposed 16 horses to two positive treatments (grooming and being presented with food) and two negative treatments (having to compete with other horses for food and having a plastic bag waved in front of them). Photographs of the horses' faces were taken throughout each treatment and presented to two impartial observers. Observers were asked to measure the number of wrinkles around each animal's eyes, and the angle between the arch of the highest wrinkle and the centre of the eye. Researchers focused on eye wrinkles because the level of wrinkling is correlated with how contracted the inner eyebrow muscles are. Results showed that the angle of eye wrinkles decreased in response to positive stimuli (mean of 10.3°) and increased in response to negative stimuli (mean of 42.5°) when compared to control photographs. The experiment's

results were also found to display high intra-observer reliability ( $ICC_{\text{average}}=0.976$ ) and high inter-observer reliability ( $ICC_{\text{average}}=0.970$ ). The findings of this article are significant as they not only suggest that horses can experience emotions, but that they can also express them through the intensity with which they contract specific facial muscles. Questions presented by this research include whether horses can depict specific emotions through their facial expressions, and how facial indicators of emotions can be used to improve their welfare.

### **Contribution**

I included this article in my review as it further establishes facial expressions in horses to be a form of non-verbal communication used to express their pain, as well as other emotions. This study advanced previous knowledge in the field of equine behaviour as it focused on the emotional experience of horses rather than their physiology. The results of this study were congruent with past research by suggesting facial indicators to be a reliable indicator of a horse's state (both emotional and physical). Future research could use these finding to determine the full range of emotions experienced by horses.

**Number of times this article has been cited: 45**

## **Method of Equine Pain Assessments Using Facial Expressions and Other Behaviours**

### **Article 6**

#### **Reference**

Dalla Costa, E., Minero, M., Lebelt, D., Stucke, D., Canali, E., & Leach, M. C. (2014).

Development of the horse grimace scale (HGS) as a pain assessment tool in horses undergoing routine castration. *PLoS ONE*, 9(3), e92281.

<https://doi.org/10.1371/journal.pone.0092281>

#### **Summary**

The objective of this article was to develop a standardized horse pain scale that would increase accuracy of assessments made and be available for use with minimal training on farm-settings. The Horse Grimace Scale is being studied as previous assessments (commonly behaviour-based) require extensive training, long observation periods, and for horses to be in severe pain. The study design used two groups of horses undergoing routine castration with anaesthesia to test the HGS post-surgery; group A (N=19) got one dose of analgesic, and group B (N=21) got two. Group C (N=6) underwent anaesthesia for a non-invasive procedure to serve as a control. A panel with equine expertise scored pre-determined facial indicators of pain on a scale from 0 (not present) to 2 (obviously present) using photos of the horses 8 hours pre-/post-procedure. Measured facial expressions included stiff ears, orbital tightening, tension above eyes, strained chewing muscles, mouth strain/pronounced chin, and strained nostrils/flattening of profile. Researchers found that the HGS offered higher average accuracy of pain assessment (at

73.3%) than pre-existing methods. High inter-observer reliability was also displayed with an Interclass Correlation Coefficient of 0.92. Thus, the HGS was found to be an effective tool in standardizing equine pain appraisal while requiring minimal training to obtain reliable results. Of the six measured facial indicators all proved easy to assess aside from eye/mouth tension and chin prominence in horses with dark coats, which were categorized as “not able to score” 12% and 16% of the time, respectively. Conversely, all other indicators were identified as such on average only 8% of the time. Moving forward, research could work to address modifications required to make the HGS more suitable for darker horses. Moreover, with the development of the HGS more opportunities arise to study effects of various procedures and medical conditions on horse welfare.

### **Contribution**

This article was chosen as it provides an overview of facial indicators of pain in horses, outlines pre-existing pain assessment methods, and tests a new assessment tool (the Horse Grimace Scale). The HGS proved effective for standardization of equine pain assessments, while increasing detection accuracy and making the skill more widely accessible for farm use. The findings of the study are congruent with previous works as HGS results were positively correlated with other methods of equine pain assessment. The study provides a new line of questioning to be explored regarding different facial markers of pain in horses of differing colours.

**Number of times this article has been cited: 395**

## Article 7

### Reference

van Loon, J. P. A. M., & Van Dierendonck, M. C. (2018). Objective pain assessment in horses (2014–2018). *The Veterinary Journal*, *242*, 1–7. <https://doi.org/10.1016/j.tvjl.2018.10.001>

### Summary

The objective of this article was to review the influx of recent (2014-2018) studies on horse pain assessment, primarily composite and facial expression pain scales. For each study included, the authors described study designs, validity, and limitations. Data were compiled using PubMed, Science Direct, Web of Science, and Google, with the following search terms: horse, equine, pain, scale, behaviour, facial expression, assessment. Researchers first identified the importance of validity in the field of equine pain assessment and proposed a two-step process that requires two independent datasets being used for statistical analysis. Singular and composite pain scales were found to mostly focus on acute causes of pain, such as acute colic or orthopedic pain. These studies had limited validity due to small sample sizes, and the need for further research to make sure results are generalizable. Studies focused on facial expression-based pain scales allow for generalizability across different types of pain, and displayed high accuracy, but also need follow-up studies to establish validity. The article also discussed pain scales for ridden horses and suggested that their results don't support use in clinical settings. The review also recognized the influence of personality characteristics on pain expression and pointed to a high risk of misinterpreting pain-scale data due to differing temperaments between horses. Researchers concluded that while many publications have been made in recent years to assess

equine pain (of which facial indicators were the most promising), they all largely require extensive efforts to validate their results. This article was published with the intent to compile recent findings in the field and identify the next steps required to develop a comprehensive pain scale for horses. The article presents questions regarding how assessments could eventually be used to study clinical efficacy of treatment techniques to further improve horse welfare.

### **Contribution**

This article was chosen as it highlights the most recent methods for pain assessment in horses, explores unique indicators of pain used in each (largely facial expressions), and how the data were analyzed. This article contributes to the field as it congregated the large influx of publications made in recent years regarding equine pain assessment scales and summarized their findings to find areas of overlap and contrast. The article findings were used to identify gaps future research needs to address before pain scales become more viable for clinical practice and ultimately be used to improve horse pain management.

**Number of times this article has been cited: 22**

## Article 8

### Reference

Mullard, J., Berger, J. M., Ellis, A. D., & Dyson, S. (2017). Development of an ethogram to describe facial expressions in ridden horses (FEReq). *Journal of Veterinary Behavior, 18*, 7–12.

<https://doi.org/10.1016/j.jveb.2016.11.005>

### Summary

The objective of this article was to develop an ethogram describing facial expressions of ridden horses that could be used reliably to assess equine pain. This study diverged from previous works as it sought to identify/assess pain specifically in ridden horses, as opposed to horses experiencing immediate pain due to illness or surgery. Moreover, this study aimed to determine indicators aside from the typical abnormalities with gait and posture which horses can easily overcompensate for and eventually conceal. Researchers first developed an ethogram by viewing footage of 150 ridden horses and describing reoccurring facial expressions they observed. While some of the identified facial expressions concerning tension of the eyes and mouth overlapped with features previously observed in non-ridden horses, researchers also noted expressions specific to ridden horses such as sticking out of the tongue, crossing of the jaw, and wrinkling of the nostrils. To test the ethogram's validity, researchers presented 13 individuals with varying levels of equine expertise photographs of 30 ridden horses that were categorized as either lame or non-lame. The developed ethogram required assessors to answer "yes" or "no" or "cannot see" to the presence of various eye, mouth, tongue, and head positions. Statistical tests performed on the assessors' results found that the ethogram displayed high inter-observer reliability with 87% agreement between assessors on the facial expressions observed in

each photograph. These findings are not only significant because they establish the first pain assessment tool for ridden horses, but also because the developed ethogram was found to be a reliable resource for use by individuals with expertise in different facets of equine management. A question raised by this research is whether it is possible to simplify the ethogram for use by individuals without equine expertise so they can accurately identify if their horse requires medical attention or not.

### **Contributions**

This article was included in my review as it expanded the scope of my research beyond facial indicators of pain in horses undergoing immediate noxious stimuli to include ridden horses that have experienced pain for years without displaying obvious indicators. This article advanced research on equine pain face as an ethogram had never been previously created for ridden horses. The developed ethogram was congruent with previous research as facial indicators of pain were determined to be a reliable assessment tool. Future research could expand the ethogram so it can be used for both horses in motions, and horses in photographs.

**Number of times this article has been cited: 49**



## Article 9

### Reference

Pritchett, L. C., Ulibarri, C., Roberts, M. C., Schneider, R. K., & Sellon, D. C. (2003). Identification of potential physiological and behavioral indicators of postoperative pain in horses after exploratory celiotomy for colic. *Applied Animal Behaviour Science*, *80*(1), 31–43.

[https://doi.org/10.1016/S0168-1591\(02\)00205-8](https://doi.org/10.1016/S0168-1591(02)00205-8)

### Summary

The objective of this article was to establish behavioural and physiological characteristics of healthy horses so that the information could be used to identify indicators of post-operative pain. While previous studies have aimed to determine indicators of equine pain, none have established a baseline for the behaviour and physiology of normal/healthy horses. The authors' goal for this research was for it to be used in the future to study effects of various horse pain-management techniques. The study design utilized twenty-seven horses split into three groups: 10 healthy control horses (Group 1), 10 horses undergoing anaesthesia (Group 2), and 7 horses undergoing surgery for gastrointestinal problems (Group 3). Behavioural and physiological data were collected every four hours for a period of twenty-four hours starting the day after Group 3 horses underwent surgery. Physiological data recovered included heart rate and plasma cortisol (stress hormone) levels. Two observers blind to the experiment used numerical rating scales and time budgets for gross behaviours (head/ear position) and responsiveness to stimuli (opening of stall doors) to collect behavioural data and ensure inter-observer reliability. Results indicated that Group 3 had higher heart rates ( $P < 0.03$ ) and plasma cortisol levels ( $P < 0.001$ ) when compared to

both Group 1 and 2. Behaviours such as locomotion and responsiveness were also found to be reduced in Group 3. While Group 3 horses spent significantly more time displaying pain behaviours than the other two groups ( $P < 0.001$ ), they were found to spend upwards of 78.6%-87.9% of their time not moving at all. These results are particularly significant as contrary to previous research, they suggest *lack of* voluntary movement to indicate pain, rather than specific facial expressions. This study raises questions regarding whether lack of movement can be generalized as a response to all pain, or just post-operative pain in horses.

### **Contribution**

This article was included in my review as it set a baseline for healthy horse behaviour and physiology. This study advanced the field of equine pain as it observed behaviour and physiology simultaneously instead of focusing on just one. This study contradicted previous works as it found lack of movement to be an indicator of pain in horses rather than the presence of pain-specific behaviours. Future research could use baselines for health established in this study to identify more indicators of pain in horses and develop new assessment tools for equine pain management.

**Number of times this article has been cited: 201**

## Article 10

### Reference

Taylor, P. M., Pascoe, P. J., & Mama, K. R. (2002). Diagnosing and treating pain in the horse. *Veterinary Clinics of North America: Equine Practice*, 18(1), 1–19.  
[https://doi.org/10.1016/S0749-0739\(02\)00009-3](https://doi.org/10.1016/S0749-0739(02)00009-3)

### Summary

The objective of this article was to review findings of research on pain assessment methods in horses and other animals between 1975-2002. This work is of significance as while there had been a surge of studies focusing on animal pain, their findings had not been consolidated to determine areas where future research needed to focus on. The authors structured their article around the two main assessment types encountered throughout their review of the literature: objective and subjective. Objective assessments mainly included analysis of heart rate, beta-endorphins (pain-reducing hormones), and catecholamines/corticosteroids (stress hormones). Such physiological parameters were not found to reliably indicate pain as they were affected by a variety of factors aside from pain. Moreover, hormone analysis was described as particularly problematic as their levels peaked 4-8 hours after pain began, meaning they could not be used to make accurate or immediate assessments. Non-physiological objective assessments such as gait analysis, ground reaction force (a test for horse lameness), and response to pressure displayed high reliability in detecting pain. However, their need for highly specialized equipment did not make them feasible for wide-spread use. In terms of subjective assessment methods, Taylor et al. (2002) found behaviour-based assessments such as numerical

rating scales and grimace scales to be not only reliable, but easy to use. A problem noted with all subjective assessments, however, is that they lack validity due to the influence of observer training and bias on interpretation of results. The conclusion reached by authors of this review was that behavioural tools showed the most promise in terms of their ability to assess pain, but that further research was required to increase their validity. A question raised by this study is how the pain assessment tools proposed by different researchers could be implemented for use outside of clinical settings.

### **Contribution**

I chose this article for my review as it consolidates pain assessment tools researched in a variety of animals and applies the information to horses. This review is important as while a considerable amount of research has been done on animal pain, very few studies have focused on equine pain specifically. By determining behavioural assessments to be the most reliable at identifying equine pain, future research can focus on ways to standardize behavior-based techniques so they can be used to improve horse welfare in non-clinical settings (such as on farms).

**Number of times this article has been cited: 135**

- My annotated bibliography was ordered into three sections to provide a layered understanding into the field of equine pain face. The first section describes what pain is, the physiological basis behind facial expressions, as well as how facial expressions can be used as a form of non-verbal communication. The second section explores equine use of facial expressions to indicate pain and other internal states (such as emotions). The third section provides an understanding for how facial expressions can be used to develop pain assessment tools, as well as including reviews on diagnostic pain tools for horses.