Pain/stress indicators in horses (*Equus caballus*)

I have divided the ten articles under different headings that include the different tools, visual studies, and external factors that affect horses that have pain in different body regions. My last heading has two articles that synthesize all the known information and provide a critical review on the subject of pain/stress indicators in horses (*Equus caballus*).

Studies on pain assessment tools in horses (*Equus caballus*)

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), 1–10. https://doi.org/10.1371/journal.pone.0092281

Summary: This article investigated the use of the horse grimace scale (HGS) as a way to better understand pain indicators in horses (*Equus caballus*) that undergo routine surgeries such as castration. Previous studies have tried to identify pain indicators in horses by looking at physiological changes and changes in behaviour. Dalla et al. (2014) aimed to develop a standardized pain scale based on the facial expressions of horses after being introduced to routine castration. There were 40 horses for the two treatment groups who underwent castration with the presence of anesthesia. Group A (n = 19) received an analgesic (pain reliever) Flunixin before the anesthesia while group B (n = 21) received Flunixin once before the anesthesia and then again six hours after the surgery. There also was a control group consisting of six horses who underwent non-invasive procedures without any surgery and were exposed to the same conditions as group A. Cameras were used to record facial changes that were used in composite pain scale (CPS), HGS scores, and behavioural changes. These were compared before and eight hours after the procedure. Both groups A and B showed a greater number of jaw clenches and grimaces eight hours after the surgery. Even though Group B received Flunixin two times and group A received it once, the number of jaw clenches and grimaces were the same for both groups post-operative surgery. These researchers mentioned that applying Flunixin twice did not significantly change the results between the groups. The study was significant as it demonstrated that using the HGS is an effective way of determining pain indicators in horses undergoing routine surgeries. Although this study provided significant results, other research experiments need to be conducted to check if HGS is relevant when performing more painful procedures.

Contribution: I included this article because it gives a better grasp of the indicators that horses (*Equus caballus*) are in pain, and it's an excellent resource for figuring out where to look for these indicators. There are previous studies that have tried to define pain indicators in horses e.g., the composite pain scale has been able to explain pain in horses by looking at specific physiological and behavioural changes but not necessarily facial changes. This study came up with some important follow-up questions e.g., how anxiety and fear could potentially affect the facial changes in horses.

Bussières, G., Jacques, C., Lainay, O., Beauchamp, G., Leblond, A., Cadoré, J.-L., Desmaizières, L.-M., Cuvelliez, S. G., & Troncy, E. (2008). Development of a composite orthopaedic pain scale in horses. *Research in Veterinary Science*, *85*(2), 294–306.
https://doi.org/10.1016/j.rvsc.2007.10.011

Summary: Previous research established that our nervous system detects pain by using receptors known as nociceptors, which send external signals to the spinal cord and brain in order to warn the individual of potential damage. Bussieres et al. (2008) investigated the development and validation of a composite pain scale (CPS) for orthopedic pain in horses. A multifactorial numerical rating CPS was developed that included physiological and behavioural parameters. Each response was given a score of 0 (absence of pain) to 3 (presence of pain). 18 horses were divided into six groups, each with three horses under different treatments. Three control groups were used: one with sedation, one with sedation and epidural placebo, and one with sedation and epidural analgesia. Three pain-induced groups were used; one with sedation and non-steroidal anti-inflammatory drug (NSAID) monotherapy, the second with sedation and pre-emptive epidural analgesia, and the third with sedation, NSAID, and epidural analgesia. The control group was recorded every hour up to 12 hours, then every 6 hours up to 24 hours, while the pain-induced group was recorded every hour up to 24 hours. The most significant behaviours recorded were palpation and change in posture while pawing on the floor, kicking at the abdomen, and head movement were some other lesser significant behaviours observed. Physiological parameters were not useful in understanding orthopedic pain in horses, according to Bussieres et al. (2008) because they did not record any changes in them. The study is significant as it provides a new tool for measuring pain in horses and it also demonstrated significant changes in the presence of preemptive multimodal analgesia, which was found to be very effective in reducing orthopedic pain in the horses tested. This study included how other movement tests on horses should be performed in order to supplement these findings and provide appropriate care for horses.

Contribution: I included this article because it provides information on an effective pain scale that can be used to measure pain in horses. This article provides an effective way to study pain by developing a pain scale that provides information on physiological and behavioral parameters. This study wanted to develop and validate a CPS in order to define orthopedic pain in horses. This study came up with some important follow-up questions e.g., further movement tests need to be carried out to supplement these results and help in taking care of horses.

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: Ridden horses may seem sound but undergo musculoskeletal problems which are noticed by trained observers and often go unnoticed by owners, riders, etc. which is why it is very important to study and prevent this pain. In order to study pain in horses, many ethograms have been developed but there are not any ethograms for ridden horses. Mullard et al. (2017) aimed to develop a facial ethogram for ridden horses and wanted to check if individuals out of the field can interpret it easily. They developed an ethogram with the help of previous publications and by using 150 photographs of lame and non-lame horses. Thirteen assessors were used who ranked each facial behaviour as 'yes', 'nor' or 'cannot see'. Tight or relaxed jaw, open mouth, salivation, and open right eye were some of the facial behaviours that were observed. As photographs were being studied, some behaviours could not be correctly seen e.g. twisted head had a greater score for 'cannot see' maybe because it was difficult to observe this behaviour in just photographs. Individuals from different backgrounds also tested these ethograms and there was no difference in the assessor's scorings compared to the trained assessors for other facial behaviours. The interrater agreement came out to be 87% which showed that the results were reliable. This research was significant as it concluded that the developed ethogram could be used to describe facial changes in ridden horses by people from varying professional backgrounds. Mullard et al. (2017) said that further research needs to be carried out that uses this ethogram to look for pain-related behaviours in ridden horses.

Contribution: This article is included because it offers a new tool - an ethogram to study facial expressions of ridden horses. An important aspect of this ethogram is that it has been validated by individuals from various fields, proving that it is a reliable tool that can be used to study pain-related facial changes in horses. For the first time, an ethogram was developed for ridden horses, as they also undergo pain. This study made some important recommendations for future research, including the need to investigate pain-related changes in facial expression in ridden horses using the ethogram developed.

Studies on visual observations of facial changes in horses (*Equus caballus*)

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: There is no universally accepted pain scale for horses; some focus on behavioural aspects while others focus on physiological aspects. Gleerup et al. (2015) aimed to define equine pain face in the presence and absence of an observer. Pain was induced using noxious (painful) stimuli including using a tourniquet (a device that applies intense pressure to limit blood flow) and using a topical Capsaicin creme (which burns when applied to the skin). Six horses were used in three different treatments; two were controls, two had tourniquet introduced on right and left thoracic limbs, and two had capsaicin introduced (one had the creme applied on the right shoulder and left thigh while the other had creme at the left shoulder and right thigh). These treatments were done twice; once with the presence of an observer and once without. Video recordings of the facial expressions were taken 20 minutes after the introduction of the treatment. The researchers used a modified version of the composite pain scale (CPS) to observe pain behaviour. Asymmetrical ears, the angled appearance of the eyes, tense stare, and tension in the lips and chin were observed in horses that underwent the painful treatments. Apart from this, an ethogram of the facial expressions was developed using the 'no observer' trials. The results of the painful treatments showed significantly greater pain scores and the researchers observed greater facial changes from the painful treatments as compared to the control treatments. Another significant finding was that in the presence of an observer the researchers observed greater attention-seeking behaviours by the horses. This article was significant as it introduced pain stimuli to observe and record facial changes in horses. Although this experiment provides significant results it also mentions that further tools need to be developed in order to grade pain more quantitatively.

Contribution: I included this article because it provides great evidence of pain in horses and these findings can be used to improve tools for pain recognition. It provides us with different facial changes that are observed when horses are in pain. The only result that did not support previous findings was that horses were seen to want more attention from the observers under pain which had not been seen before. This study came up with some important recommendations for future studies e.g. researchers stressed developing other pain measuring tools to provide better evidence for pain in horses.

Merkies, K., Ready, C., Farkas, L., & Hodder, A. (2019). Eye blink rates and eyelid twitches as a non-invasive measure of stress in the domestic horse. *Animals*, *9*(8), 1–10.
https://doi.org/10.3390/ani9080562

Summary: Stress is defined as external stimuli that have an effect on an individual's internal environment. Non-invasive testing of spontaneous blink rate has been used to measure stress in humans but hasn't been done on horses. Spontaneous blinks are different from voluntary and reflex blinks as they provide information on attention and working memory. Merkies et al. (2019) set out to investigate the spontaneous blink rate in horses (*Equus caballus*) in order to assess stress after being exposed to external stimuli. The 33 horses were randomly placed in four treatments. First was the control treatment in which horses were exposed to their normal environment, the second was feed restriction treatment in which the researchers withheld feed at regular feeding times, third was a separation treatment in which horses were separated from their mates, and fourth was the startle test treatment in which a ball was suddenly thrown in front of the horse. The researchers used video cameras, ethograms, and heart rate monitors to record the eyelid movements, behavioural changes, and heart rates. After the appropriate treatments were introduced the horses were observed for three minutes each. This study found some significant results in terms of eyelid twitches and heart rates. Feed restriction was suggested to be the most stressful stimulus, as the horses' heart rates, restless behaviours, high head positions, and high eyelid twitches were all increased as compared to the control treatment. The researchers also observed no increase in heart rates and behavioural changes in the separation and startle test treatments when compared to the control treatment. They attributed these results to the horses' lack of stress in their treatment. Although this study yielded significant results, the researchers stressed the importance of further research to determine whether spontaneous blink rate and eyelid twitches vary over different time spans.

Contribution: I included this article because it provides great evidence of indicators of pain in horses and these findings can be paired with previous findings to better understand pain. This is the first study to look into the significance of eyelid twitches in horses. It also provides us with different behavioural changes and heart rate variations. This study made some important recommendations for future research, such as the need for more experimentation to look at eyelid twitches over time so that the findings can be verified and used in clinical research for the benefit of horses.

Studies that compare physiological and behavioural changes in horses (*Equus caballus*) undergoing pain in various body regions

Mayaki, A. M., Abdul Razak, I. S., Adzahan, N. M., Mazlan, M., & Rasedee, A. (2020). Clinical assessment and grading of back pain in horses. *Journal of Veterinary Science*, *21*(6), 1–10. https://doi.org/10.4142/jvs.2020.21.e82

Summary: Horses are negatively affected by back pain (BP) because it affects their abilities to perform athletically and affects their movement when riding. Diagnosing back pain in horses is challenging because it is a situation not presented by visible signs. Mayaki et al. (2020) aimed to use clinical features to classify equine back pain. Methods of diagnosis included visual inspection, palpation (feeling with fingers or hands), and examination of exercise. The sample included 24 horses with back pain and ten healthy horses. These horses were graded on a scale of zero to five for the following factors; pain response, muscular hypertonicity (increased stiffness or tightness in the muscles), thoracolumbar joint stiffness, and overall physical dysfunction (problems with movement or coordination). Digital palpations devices and reflex tests helped in grading these responses. Significant differences were seen between the horses experiencing back pain when compared to the ones that were not experiencing back pain e.g. the researchers reported higher scores for pain response, muscular hypertonicity, thoracolumbar joint stiffness, and physical dysfunction in horses with back pain. This study is significant as it provides evidence for back pain and it shows that back pain can be observed by studying palpation, back muscle hypertonicity, and cumulative grading score. The researchers mentioned that these results are very crucial in understanding back pain behaviours in horses as they not only provide behavioural knowledge but also provide useful physiological evidence. They emphasized that the findings of this research coupled with other researches have the potential to provide a better understanding of back pain behaviors.

Contribution: This article is included because it offers new information on characterizing back pain in horses effectively. There was no grading system that could be used to measure back pain in horses that is why Mayaki et al. (2020) wanted to test different behavioural and physiological parameters. The grading system would allow veterinarians to differentiate back disorders which would be helpful for treatment and preventative measures of horses. This study made some important recommendations for future research, for example using these results to compile previous research and try to come up with better treatments of horses with back pain.

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

Summary: Quantitatively measuring physiological and behavioural changes has helped evaluate pain in horses in the past but has not been used to help in the treatment of horses undergoing celiotomy (incision of the abdomen). Pritchett et al. (2003) aimed to define physiological and behavioural changes of normal horses and horses undergoing celiotomy (painful abdomen surgery) in order to see how these would change in the presence of analgesic medication (a drug that relieves pain). 27 horses were divided into three groups; ten were assigned to the control, ten were given anesthesia for a non-painful procedure and seven had to undergo celiotomy without anesthesia. The researchers recorded data on physiological and behavioural parameters at different intervals; 0,4,8,12,20,24 and 30 hours after carrying out the respective treatments. Heart rate, respiratory rate, and cortisol levels were the physiological parameters recorded. They used a numerical rating scale (NRS) to visually record behavioural changes in the three groups. Significant physiological recordings included higher levels of cortisol and elevated heart rates for the surgery group when compared to the control and anesthesia groups. The control and the anesthesia group had the same recordings for each parameter. Apart from that, the control and the anesthesia group showed more locomotion than the surgery group. Lastly, the surgery group showed more pain-related behaviours such as flehmen, flank gestures kicking at abdomen, stretching the body, etc. as compared to the control and the anesthesia groups. The researchers suggested that reduced locomotion, increased cortisol levels, and increased heart rate are potential indicators of pain in operated horses. Because the researchers obtained significant results for this experiment, they recommended additional research to specifically study whether the introduction of anesthesia in two groups of horses undergoing surgery would result in differences in behavioural and physiological parameters.

Contribution: I included this article because it tried to include various behavioral and physiological indicators that a horse (*Equus caballus*) gives off when in pain after surgery. There has been no research on pain indicators for horses undergoing celiotomy, so this research provides significant results and is important to the field. The article defines significant behavioral and physiological parameters for horses undergoing surgery and how they differ from normal horses. It recommends conducting research on postoperative horses and checking whether the introduction of anesthesia affects the physiological and behavioral parameters between them.

Studies that look at external factors that may induce stress/pain in horses (*Equus caballus*)

Lundblad, J., Rashid, M., Rhodin, M., & Haubro Andersen, P. (2021). Effect of transportation and social isolation on facial expressions of healthy horses. *Plos One*, *16*(6), 1–17. https://doi.org/10.1371/journal.pone.0241532

Summary: Horses are known to exhibit a variety of facial changes in response to pain or stress. External changes in their situations, such as transportation, competition, and separation from the herd, can cause stress in horses. However, external changes are not the only ones that cause stress in horses; internal changes also play a part. In mammals, for example, the adrenal medulla and hypothalamic-pituitary-adrenal cortex are activated in presence of stress or pain, resulting in increased blood pressure, temperature, heart rate, and respiratory rate. Lundblad et al. (2021) wanted to look at the facial expressions of horses who had been transported and isolated. Short-term transportation (for 20 minutes) was used on 28 horses, and ten of them were also subjected to short-term social isolation (for 15 - 30 minutes). A body-mounted heart monitor was used to measure heart rate, and video cameras were used to observe all of the horses' facial changes before and after treatment. Both treatments caused an increase in heart rate. Both isolation and transportation treatments resulted in a significant number of facial changes. Flared nostrils, repetitive mouth behaviours, increased eye white, tongue show, and ear movements were among the changes in facial characteristics. The findings are significant because several of these features, such as dilated nostrils, increased eye whiteness, and inner brow raiser, can be used as pain indicators for horses in pain assessment tools. Some of the facial changes, such as upper lid raiser and half blink, were only observed in horses undergoing transportation, as environmental factors such as wind may have an effect on these behaviours. These two were not observed in the isolation group because there were no environmental factors present, such as wind. The researchers emphasized the importance of further research into the relationship between facial expressions and pain in order to aid with clinical research.

Contribution: I included this article because it provides indicators of stress in horses undergoing external changes such as transportation and isolation. This article provides an effective way to study stress as various facial changes were observed when the horses were exposed to external changes. These provide information on how stressful situations such as transportation and isolation cause behavioural changes in horses. This study raised some important follow-up questions, such as the need for additional tests to investigate the relationship between facial expressions and pain/stress in horses.

Studies that assemble previous research on stress/pain indicators in horses (*Equus caballus*) and provide a critical review of the methods used

Ashley, F. H., Waterman-Pearson, A. E., & Whay, H. R. (2010). Behavioural assessment of pain in horses and donkeys: Application to clinical practice and future studies. *Equine Veterinary Journal*, *37*(6), 565–575. https://doi.org/10.2746/042516405775314826

Summary: While there are many pain expression studies on humans, there aren't as many on horses, owing to the difficulty of sampling and developing efficient approaches to evaluate pain signs. Ashley (2010), summarizes the studies of 99 research articles that reviewed pain indicators in horses and donkeys by looking at pain scales and behavioural measures such as posture changes. There were different behaviour changes for each pain region of the horses. Aggression and anxiety were some non-specific behavioural indicators seen after surgery e.g. reluctance of the horses when being handled was observed. Pain scale scoring was used to quantitatively describe whenever a behaviour change was observed. Horses' abdominal pain was observed when they rolled or kicked at their abdomen. Pain in the limbs was recorded when the horse was observed shifting its weight. Head shaking was observed as a sign of head pain. The paper also discussed physiological changes such as a faster heart rate, which indicated that the animal was in discomfort post-surgery. Studies on the impact of painkillers on horses and donkeys were also mentioned, with the results indicating that using painkillers aided with pain relief because the animals showed fewer signs of distress. Ashley (2010) effectively described all the visible behaviour changes in horses under pain in various body regions. Although significant results are reported, the paper emphasizes the need for more research into the degree of pain and determine if these changes in behaviour on horses were due to pain alone or fear. Prey species like horses and donkeys have evolved to mask signs of pain for their own protection against predators so it is important to check how and if these results would be different in the presence of a predator.

Contribution: I included this article as it attempted to include various behavioural and non behavioural indicators that a horse (*Equus caballus*) gives off when in pain. The article is the first to define each behavioural change that occurs when the horse experiences pain in different areas of the body. While supporting most of the results of previous papers, it also emphasizes the need for experiments that measure intensity and fear in animals when observing pain. This review provides an excellent summary of previous research evaluating pain in horses that can be used in future research and to improve current clinical practice.

Hausberger, M., Fureix, C., & Lesimple, C. (2016). Detecting horses' sickness: In search of visible signs. *Applied Animal Behaviour Science*, *175*, 41–49.
https://doi.org/10.1016/j.applanim.2015.09.005

Summary: Although it is clearly challenging to determine if a horse is in pain, owners and researchers need to understand when this happens for the welfare of the animal. Sometimes the horses exhibit visible signs of discomfort, while other times they hide these signs, so studying physiological changes is also important. Hausberger et al. (2016) conducted a critical review of 94 articles to determine whether or not effective tools for quantitatively assessing pain in horses exist. They mentioned how sickness in a horse can range from mild discomfort to acute pain. And that suffering in horses is widely underestimated because people are not aware of the indicators. Various qualitative and quantitative behavioural changes were observed across a number of experiments. Some of the tools used were the composite pain scale (CPS) was used to observe behaviours such as head and neck postures, kicking at the abdomen, and so on, and the horse grimace scale (HGS) was used to observe facial changes such as mouth strains, and orbital tightening. According to Hausberger et al. (2016), while all of these pain scales produce significant results, they lack clear definitions because these studies are solely based on visual learning. Aside from these, extensive research has been conducted on behaviours observed in horses suffering from pain or stress, such as ear position, stall orientation, and aggressiveness. According to the paper, healthy horses are attentive and ready to respond to stimuli, so a lack of responses may indicate that the horse is ill. Although significant advances have been made that will undoubtedly assist owners in recognizing when their horses are in pain, more research is required to reach an agreement on pain indicators.

Contribution: I included this article as it attempted to include various behavioural tools that have been and are being used to study pain in horses. The article lists different behaviors that pain scales measure by observing horses in real-time. This article stresses the importance of the results of previous papers but also emphasizes the need for further experiments that consider physiological and behavioral factors. This review provides an excellent review of previous research evaluating pain in horses, which can assist owners in recognizing when their horses are in pain and researchers in improving clinical practice.