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Ingrid Lindstedt

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Facial expression of pain in horses undergoing flexion test and relation to lameness

En studie av hästens ansiktsuttryck för smärta i samband med böjprov och relation till hälta

Ingrid Lindstedt

Supervisor: *Pia Haubro Andersen, Department of Clinical Sciences, SLU*

Examiner: *Marie Rhodin, Department of Clinical Sciences, SLU*

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SUMMARY

Recent studies have indicated that horses in pain exhibit a facial expression of pain. The so called pain face includes a number of different pain specific attributes. Up until now, the equine pain face has only been studied on horses with induced somatic pain or post-operative pain. Subsequently this study aimed to investigate whether the pain face can work as clinical tool in pain assessment of horses undergoing flexion tests in lameness diagnostics. The hypothesis was that horses with positive flexion tests would show pain face during the flexion, whereas horses with negative flexion tests would not show pain face during the flexion.

24 horses were included in the study. All horses showed positive flexion test reactions from at least one joint. The horse faces were filmed during flexion tests as well as when they were not provoked. Three film sequences for each horse was selected for further investigation: a negative flexion test, a positive flexion test and a control sequence. All film sequences were randomized and scored by blinded observers. The observers scored each facial expression as having no pain face, a pain face present or an intense pain face present.

No significant difference in the grading of pain face could be seen when comparing positive versus negative flexion tests. The same went for comparing positive flexion tests versus control sequences. The reason for these non-significant findings can be multiple. There might be large individual variations in the expression of pain face in different situations. The result could also indicate that there were several different pathological (and painful) conditions in these horses (chronic or non-chronic) that influenced the face mimic of the horses. Another possible cause for the result could also be that the equine pain scale does not apply very well to horses with orthopedic conditions.

SAMMANFATTNING

Nyligen utförda studier har visat att hästar som befinner sig i smärta visar ett ansiktsuttryck förknippat med smärta. Detta ansiktsuttryck inkluderar ett antal smärt-specifika ansiktsdrag. Fram tills nu har det ekvina ”smärtansiktet” enbart studerats på hästar med inducerad somatisk smärta eller postoperativ smärta. Denna studie syftade till att undersöka huruvida ”smärtansikte” kan användas som ett kliniskt verktyg i bedömningen av smärta hos hästar som genomgår böjprov som en del av hältdiagnostiken. Hypotesen var att hästar med positiva böjprov skulle visa ”smärtansikte” under böjningen, medan hästar med negativa böjprov inte skulle visa ”smärtansikte” under böjprov.

24 hästar inkluderades i studien. De uppvisade alla positiva böjprovsreaktioner från minst en led. Hästarnas ansikten filmades både under böjprov samt en gång då hästarna inte provocerades på något vis. Tre filmsekvenser valdes ut för varje häst för vidare undersökning: ett positivt böjprov, ett negativt böjprov samt en kontrollsekvens. Alla filmsekvenser randomiserades och bedömdes av observatörer som var blindade för böjprovsresultatet. Obesrvatörerna smärtbedömde varje sekvens utifrån hästens ansiktsuttryck (inget ”smärtansikte”, ”smärtansikte” närvarande eller intensivt ”smärtansikte”).

Ingen signifikant skillnad av graderingen av ”smärtansikte” kunde observeras när man jämförde positiva och negativa böjprov. Detsamma gällde när man jämförde positiva böjprov med kontrollsekvenserna. Anledningarna till dessa icke-signifikanta resultat kan vara många. Det kan vara så att det finns stora individuella skillnader i uttrycket av ”smärtansikte” i olika situationer. Resultatet skulle också kunna indikera att det finns flera olika patologiska (och smärtsamma) faktorer (kroniska och icke-kroniska) som påverkar hästens ansiktsmimik. En möjlig orsak till resultatet kan också vara att denna smärtskala inte går att applicera särskilt väl på hästar med ortopediska smärtsamma tillstånd.

CONTENT

INTRODUCTION

Lameness is the most frequent cause for a horse to visit the veterinarian (Penell, 2009). This makes lameness diagnostics one of the major fields of equine veterinary medicine. At the same time, the frequency of use places great demands on the methods and diagnostics used.

However, the greatest challenge for the horse practitioner might be that the horse cannot speak: it cannot tell us where the pain is originating from. Much effort is therefore put into localizing the source of pain in each case. In the end lameness diagnostics is all about evaluating all the clues and pieces of information that you can receive through medical history, clinical examination and visual exam at exercise (Stashak *et al*., 1987). Lameness diagnostics is therefore an important area to develop and refine as to make it more precise and objective. One part of this puzzle might be the development of the so called "lameness locator", which was created as to objectively tell the practitioner which leg of the horse is lame (Keegan, 2004). Another part of the puzzle could be making flexion tests more objective, by standardizing the time and force used (Keg *et al*., 1997).

Several recent studies have been focusing on the facial expression of pain in animals such as mice (Langford *et al.*, 2010), rats (Sotocinal *et al.*, 2011) and rabbits (Keating *et al.*, 2012). There are also two publicized studies regarding pain face in horses (Gleerup *et al.*, 2015; Dalla Costa *et al.*, 2014). Using pain face in the horse as a pain assessment tool is, though, not yet validated, but could also become an important factor to evaluate during lameness examinations.

The aim of this study was to investigate whether the pain face can work as clinical tool in pain assessment of horses undergoing flexion tests in lameness diagnostics. The hypothesis was that horses with positive flexion tests would show pain face during the flexion. At the same time horses with negative flexion tests would not show pain face during the flexion.

LITERATURE REVIEW

Pathology of joint-associated diseases

When a clinician is performing a flexion test of one or several joints, the intention is most often to reveal or rule out some sort of joint-associated disease or injury (Meijer *et al.*, 2001). When speaking about joint-diseases, it is often traumatic arthritis that is referred to. Traumatic arthritis involves a collection of different pathological and clinical states which has been summarized in the list below:

- Synovitis (inflammation only in the synovial membrane)
- Capsulitis (inflammation in the joint capsule)
- Sprain (injury of a joint-associated ligament)
- Intra-articular fractures
- Meniscal tears (only when speaking about femorotibial joints)
- Osteoarthritis (when the articular cartilage is affected by degradative changes)

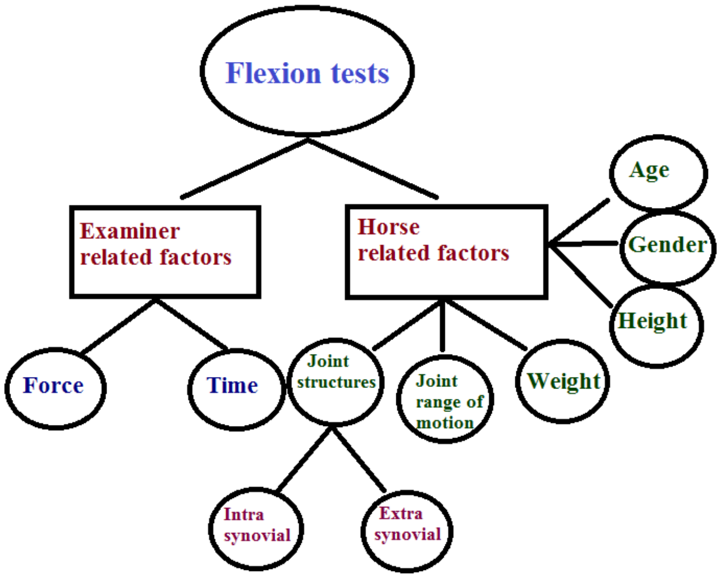
All of these conditions are more or less painful and the pain is dependent on stimuli acting on mechanoreceptors (nociceptors, see below) on the different affected structures either by direct stimuli or via a wide variety of different mediators such as prostaglandins, inflammation mediators etc. (Stashak *et al.*, 1987).

The flexion test

The flexion test as a clinical tool in lameness diagnostics has been used at least since Hertwig first mentioned it in 1850. He then used it to detect bone spavin in the hock (Hertwig, 1850). The flexion test as a clinical tool has since then developed and been refined throughout the years. Nowadays clinicians normally use the flexion test for two different reasons. One is to intensify the pain from a joint (or a joint related structure) when looking for a cause for lameness. The other area of use is when looking for a potential subclinical joint-related problem in a pre-purchase or insurance examination (Stashak, 1987; Goble, 1992). The theory behind the test is that by provoking tissues and structures inside and outside the joint, a clinical, or subclinical, pain will be intensified and visualized as a lameness (or intensified lameness).

There are a large number of factors influencing the outcome of a flexion test. These can be divided into examiner-related factors (such as force and time) and horse-related factors (either physiological or pathological). Some research has been done on these fields, even though there is more to be done. One could for example hypothesize that the size of the examiner influences the outcome of the flexion test, since this could give rise to, for example, different angles in carpus when Mc3 is pulled in a more or less lateral direction when flexing the proximal joints of the front leg. No research has been done on this, though. The different factors are summarized in figure 1 below. The figure gives only some examples of the different factors and does not attempt to be complete.

Figure 1. *Examiner and horse related factors*



Examiner related factors

When it comes to the examiner related factors, the advised time and flexion to be applied is very poorly defined. Many publications don't even mention what force they find appropriate and in many publications where the force is defined, it is not motivated. Advised time varies from 30 seconds up to 3 minutes and advised force varies from 100 N up to 300 N in some different publications (Goble, 1992; Keg *et al*., 1997; Keller, 1983; Pollitt, 1995; Verschooten 1990; Verschooten & Verbeeck 1997). In these publications it has been stated that different clinicians use a wide variety of the amount of force applied when they perform the flexion test.

Vershooten & Verbeeck performed a study where they used an objective tool to measure the force applied by the clinician during a flexion test. The aim of the study was to determine the optimal force to be used. According to this study the optimal force and time was 100 N during 1 min. This conclusion was based on the force and time when the least false positive reactions appeared (high specificity). Nothing was mentioned about the sensitivity (the amount of false negative results) when performing it in this way. According to Stashak, though, it is more common with false positive results than false negatives and false positives are most common in the front fetlock (Stashak, 1987 p. 143). In the study of Vershooten & Verbeeck they also found that the amount of positive flexion tests dramatically increased when force and/or time increased. A force of 150 N for 3 minutes resulted in ten times more positive results than that of 100 N for 1 minute (Verschooten & Verbeeck, 1997).

Keg and his researchers concluded that there is a large variation between different clinicians in the amount of force applied. In a group of 27 clinicians the mean force used was 145 N. The force applied ranged from 37 up to 113 percent of the mean force. This makes the flexion test, if not standardized by force and time, very hard to use as a tool when comparing the result of different clinicians. Another interesting observation made was that females tended to generally use 20% less force than males (Keg *et al*., 1997). Such a conclusion is in contrast, though, to the results of Verschooten & Verbeeck: they found that females used a mean force of 190 N and males used a mean force of 131 N (Verschooten & Verbeeck, 1997). In this study no comments were made on whether the difference was statistically significant or not. In both studies the flexion tests were limited to the distal forelimb.

With the aim to investigate whether it is possible to standardize the force applied by the clinician during a flexion test, a study was made 2016 by Giusto and his researchers. A pressure-sensitive glove was evaluated for this cause. The result of this study was regardless of whether the clinician was experienced or not the glove could easily be used to standardize the force during the test. A factor that cannot easily be controlled, however, is that the horse may move during the flexion test and thus cause loss of pressure for shorter periods of time (Giusto *et al*., 2016). As in the previous studies mentioned above, the flexion tests were limited to the distal forelimb.

In one study the aim was to evaluate whether the result of the flexion test is different when the time is set to 5 respective 60 seconds of flexion. This study was performed only with proximal hind limb flexion. The conclusion was that it was more likely to get a positive result if flexing the leg for 60 seconds compared to flexing it for 5 seconds (Armentrout *et al*., 2012). This result is in line with that of the above described study by Verschooten & Verbeeck.

Horse related factors

When it comes to the horse related factors, the research is not as extensive as for examiner related factors. Two studies have been performed regarding which structures are involved and affected in the flexion test of the distal forelimb, one regarding extra synovial structures and one regarding synovial structures. Another study has also been produced on the effect of age, gender, weight, height and fetlock range of motion when it comes to flexion of the distal forelimb. When examining all of these factors, the study population in all three studies were clinically sound, non-lame horses. This means that the results of these studies are limited, and probably most applicable, to the prepurchase or insurance examination.

In the studies regarding anatomical structures affecting the outcome of the flexion test in the sound, nonlame horse, it was concluded that the most affected area while performing flexion test of the distal forelimb is the fetlock and its surrounding structures. The structures distally to the fetlock joint only affects the result minimally. This was concluded by performing flexion tests before and after various nerve blocks (Kearney *et al*., 2010) and intrasynovial anaesthetic blocks (Meijer *et al*., 2001).

In the study regarding age, gender, weight, height and fetlock range of motion, the force used was 150 N for 1 minute when the flexion tests were performed. 60% of the study population (that consisted of sound, non-lame horses) showed a positive reaction (to some extent) to the flexion test. The authors comment on this by writing that this fact "undermines the commonly held opinion that a positive flexion test in a non-lame horse is an indication of a potentially dangerous subclinical problem". The two factors that significantly influenced the outcome of the flexion test were age and gender. Mares showed positive flexion tests in a larger extent than geldings. There was also a positive correlation between increasing age and positive flexion test. No correlation could be seen between height, weight or range of motion of the fetlock joint and the outcome of the flexion test (Busschers *et al*., 2001).

The research within the field of flexion test is, to sum it up, very limited. There is no gold standard for how the flexion test should be performed and the sources of error are many. Neither sensitivity nor specificity is very high when performing the test without measuring the force or time. In addition, the variability is large regarding both performance and evaluation of the flexion test.

Pain physiology

Pain in general

There are two different main types of pain. These are neurogenic pain and nociceptive pain (Carlsson *et al*., 1994). The neurogenic pain is initiated in other parts of the pain pathways than in the free nerve endings. Examples of neurogenic pain are phantom pain and sciatic pain. As this type of pain could be assumed not to be induced during a flexion test, the continued focus of this section will be on nociceptive pain. Furthermore, nociceptive pain can be classified into two different types – somatic pain and visceral pain (often associated with medical conditions such as colic, which is not the focus of this study) (Greenwood-van Meerveld *et al*., 2015).

The somatic nociceptive pain, that pertains to the current study, can be either superficial (when it has to do with superficial dermis and muscles) or deep (when it has to do with for example bones, joints etc.). Nociception arises when nerve impulses are initiated in sensory nerve fibers, which have free nerve endings containing nociceptors. These nociceptors are receptors which are activated by noxious stimuli. Impulse conduction can be fast or slow in this type of fibers depending on the extent of myelination. The more myelin – the faster conduction. The pain fibers react to many different noxious stimuli, such as extreme temperature and strong chemical or mechanical stimuli. The nociceptors can react directly to stimuli from a tissue-destroying process (tearing, crushing etc.) but most often they react to chemical substances which are released by the injured tissue. Examples of such substances could be prostaglandin, histamine, various enzymes etc. A common cause of deep somatic nociceptive pain is ischemia (Sjaastad *et al*., 2010). When nociceptive signals are processed in certain centers of the brain, these may finally be perceived as pain. In close association to the centers are memory centers and centers processing other sensory input, such as smell and vision. Emotions, memory and direct sensory output may therefore influence the final perception of pain (Wagner, 2010).

Pain associated with flexion tests

There are a large number of possible causes/origins for pain when a flexion test is performed. The causes/origins can be divided into intra-articular causes (including joint capsule, subchondral bone, joint cartilage, synovia) or extra-articular causes (including all the tissues surrounding the joint such as tendons, ligaments, muscles, skin etc) (Verschooten & Verbeeck, 1997).

In the publication by Stolk and his researchers, it is stated that the intra-articular pressure is markedly increased during the forced flexion of a fetlock joint (more than 45°, which happens during a flexion test). This happens both by conformational changes in the joint during the flexion and by effusion of synovial fluid. This will cause the joint capsule to be stretched and the intra-osseous pressure will increase (Stolk *et al*., 1994). It should be noted that in case of a joint disease, the intra-articular pressure might already be increased before the flexion test by pathological volumes of synovia, which might intensify the effect of the flexion test (Levick, 1979, 1983; Strand *et al*., 1998). The increased pressure causes the nociceptors in the joint capsule and subchondral bone to react and signal pain. According to Todhunter, nociceptors in pathological joints have lowered thresholds and are therefore more easily activated. However, in this study, Todhunter also states, that 2 or 3 abnormal steps after the flexion might be physiological, since the tension during the test is non-physiological (Todhunter, 1990).

Apart from the direct effect from the increased intra-articular pressure, it has also been suggested that the pressure causes obstruction of the vessels in the joint capsule and the synovial layer. This, in its turn, causes an oxygen debt, which gives rise to an ischemic pain (Busschers *et al*., 2001).

Apart from the joint-related factors, there will be a mechanical tension in the structures dorsal/cranial to the joint in question and at the same time a compression to the structures palmar/plantar/caudal to the joint in question. The combined mechanical tension and compression will cause increased mechanical stress to the nociceptors in the structures if these are in some way pathological and painful. Examples of extra-articular structures are tendons, muscles, ligament and skin (Meijer *et al*., 2001, Dyce *et al*., 2010).

Finally, there is a large number of structures involved in the flexion test, both inside and outside the joint. The result of the test gives a hint of which area is painful, but it says very little about what precise structure is causing the lameness. According to Verschooten & Verbeeck it is important that the flexion test is performed in a correct way, since false positives can occur if traction is given to, for example, the collateral ligaments if the foot is pulled in a lateral or medial direction. Such traction will cause non-physiological mechanical stress to the ligaments, which will respond with pain (Vershooten & Verbeeck, 1997).

Pain assessment

Since we cannot measure the subjective experience of pain in an animal, we have to rely on the value judgement of behavioral and physiological response to different stimuli when evaluating pain experience in an animal (Molony *et al*., 1997). This clinical judgement puts high demands on the veterinary practitioner´s ability (and professional experience) to interpret the signs that the animals are sending out. Factors such as intensity, duration, frequency and quality of the noxious stimuli should be taken into consideration when evaluating the pain experience of an animal. Additional factors such as species, breed, environment and individual differences also influences the expression of pain, which makes it even more of a challenge to estimate the amount of pain that an animal is experiencing (Flecknell, 2000: see Bussières *et al.*, 2008 p. 294)

Pain face

In humans, facial expression is considered to be the most prominent way to communicate emotional affect (Rinn, 1984). Furthermore, studies on humans as well as rabbits have illustrated that when someone is asked to assess whether a human or an animal is in pain, the eyes naturally focus on the face of the human or animal being studied (Leach *et al.*, 2011; Williams, 2002). Other studies have also suggested that humans have an evolutionarily developed skill to detect emotions by facial expression (Deyo et al., 2004).

Nowadays, the facial expression of pain is an often used tool in pain assessment and pain management when it comes to for example infants (Prkachin K., 2009; Jordan *et al*., 2011). Recently, a computer vision system was developed to detect the emotions expressed in the faces of humans (Bartlett *et al*., 2014). This system is so sensitive that one study illustrated how it, into a very high extent (higher than the human observers), could distinguish between genuine and faked emotions that are expressed through facial expression (Bartlett *et al*., 2014).

Several pain face studies have performed on various laboratory animals such as mice, rats and rabbits, leading to development of grimace scales on the specific species in focus (Langford *et al*., 2010; Sotocinal *et al*., 2011; Keating *et al*., 2012). Some recurrent pain-related features that are described in all three species are: tightening of the orbita, whisker change and changing of the ear position. Apart from these features, there are studies on species-specific pain-related features such as cheek flattening (rabbit and rat) and cheek bulge (mice) (Langford *et al*., 2010; Sotocinal *et al*., 2011; Keating *et al*., 2012).

The equine pain face

When it comes to equines, the research on this area is very recent and not very extensive. In 2011 Love and his researchers used kinematic analysis to detect that certain facial expressions changed in horses when they were exposed to injections (Love *et al*., 2011). This finding has later been validated in more recent studies (Dalla Costa *et al*., 2016; Gleerup *et al*., 2015).

Within the last few years, there are two research groups from different parts of the world that have described one similar grimace scale each almost simultaneously. Dalla costa and her team performed one study in 2014 where horses that underwent normal routine castrations were subsequent filmed post-operatively and then compared with a group of control horses. Six facial action units (elemental facial movements) were noticed - stiffly backwards ears, orbital tightening, tension above the eye area, prominent strained chewing muscles, mouth strained and pronounced chin and strained nostrils and flattening of the profile. This study lead to the Horse Grimace Scale (HGS) (Dalla Costa *et al*., 2014). The horse grimace scale has then later on been further evaluated by applying it to horses with acute laminitis to see if it is possible to assess the pain of these horses with this scale. The scores that the horses were given from the grimace scale were then compared with the scores of the Swedish Obel grade scale (the most widely accepted scale for grading the severity of laminitis) (Menzies-Gow *et al*., 2010; Viñuela-Fernández *et al*., 2011). It was concluded that the horse grimace scale is a potentially effective method for assessing the amount of pain in horses with acute laminitis as the horses with high scores in the grimace scale also showed high scores in the Obel grading scale (Dalla Costa *et al*., 2016).

The other research group (Gleerup *et al*., 2015) performed a study where horses were filmed at the same time as they were exposed to noxious somatic stimuli. The faces of the horses were then evaluated with focus on alterations in their facial expression. The facial action units were then summarized as following: “low and/or asymmetrical ears, an angled appearance of the eyes, a withdrawn and/or tense stare, mediolaterally dilated nostrils and tension of the lips, chin and certain mimetic muscles” (Gleerup *et al*., 2015). All of these facial action units were not present at all times when a horse was exposed to noxious stimuli, but alterations in their faces were always observed in greater or lesser extent.

Something that Gleerup and her researchers emphasizes in their study is that it is of importance to distinguish between the facial action units that actually attributed to pain and those that are attributed to stress, analgesics, anesthetics or other influencing factors such as human contact (Gleerup *et al*., 2015; Love, 2009; Seibert *et al*., 2003; Ashley *et al*., 2005).

It is well known that humans have two different motor systems to control facial movement. There is one subcortical extrapyramidal pathway that control spontaneous facial movements and one cortical pyramidal pathway that control voluntary facial movements. This pyramidal system allows humans to fake facial movements to express something that is not actually experienced (Kunz *et al*., 2011; Rinn, 1984; Ekman *et al*., 1982). One could then hypothesize that the same goes for horses so that if they, for some reason, would like to show humans, or other animals, a fake expression they could do so. In the study of Gleerup and her team, it was investigated whether the horse changed its facial expression during noxious stimuli when an observer was present versus when an observer was not present. The conclusion was that the face expression of the horse did not change (Gleerup *et al*., 2015). This was, though, a study with only two types of acute noxious stimuli and the observer did not interact with the horse. Finally, one could argue that even though the observers of the study did not notice any change of facial expression of the horses, it does not exclude the presence of such changes.

MATERIALS AND METHODS

Study design

The study was designed as an observational case study. Video recordings of the faces of horses that underwent flexion tests were produced. In regard to horse patients, the study included both horses that were initially lame and those who were not.

Each horse was filmed each time it had a leg flexed in addition to being filmed when there was no provocation performed, to use as a control recording. Thereby the horses acted as their own control in this study. The horses were filmed during the whole flexion test. Some horses were flexed only in specific joints and some were flexed both proximally and distally in all four legs. This means that the number of films varies between two and nine for each horse.

Inclusion criteria

- Patients that came to Löberöds horse clinic for a lameness examination. This includes initial lameness examinations, revisit examinations (2-8 weeks after another lameness examination) and regular check-ups.
- The patients had to have positive flexion test reactions from one or several joints.
- Horses where filming of at least two flexion tests (where one was positive and the other was either negative or of significantly lesser intensity) and one control recording (no provocation) was obtained.
- Patients that, in addition to being examined with flexion tests, were evaluated when trotting either on a straight line or at the lunge (or both). All evaluations had to be performed by the same veterinarian.

Exclusion criteria

- Patients that received sedation before examination.
- Patients that were under medical treatment or had been anesthetized in a joint or via a nerve the same day as the examination.
- Patients that did not show any positive flexion test reactions.
- Patients that were filmed, but where the faces of the horses could not be seen clearly enough to be able to score the films.
- Patients that the owner knew had been lame for more than three months (12 weeks). This limit was based on human medical literature describing time intervals used to divide non-chronic patients from chronic ones (Egli *et al.*, 2015; Guimaraes-Pereira *et al.*, 2016).

Animals in the study

All the owners of equine patients that came to the clinic for a lameness examination during the period of 13th of September until 4th of October 2016 were asked to participate with their horse. All the horses of the owners who said yes (only one horse owner said no) and signed a consent form were subsequently filmed. This resulted in the filming of 47 patients. Those horses that fitted into one or several of the exclusion criteria were later excluded from the study. This resulted in a total of 24 horses that were included in the study. Fifteen of the patients came for initial lameness examinations, five of the patients came for a revisit examination and four of the patients came for a regular check-up.

Procedure of lameness examination

Generally, the procedure of the lameness examination went as follows. The horse was first walked and then trotted in a straight line to detect any initial lameness. Then the horse was trotted on the left and right lunge. The lameness was estimated based on a scale of 0-5 where 0 is not lame at all and 5 is non-weight-bearing lame (according to the lameness scale of Amercian association of Equine Practitioners). Subsequently the horse underwent several flexion tests. The flexion tests were always fractionized so that either the proximal or the distal joints were provoked. Most often the horse underwent a total of eight flexion tests (one proximal and one distal for each leg). Sometimes, if the horse was only, and clearly, lame in one leg (or if it was a revisit), the flexion tests were limited to only front- or back legs or only proximal or distal flexion tests.

Description of proximal flexion test front leg: The veterinarian stands beside the horse and grabs the metacarpus of the leg and pushes it upwards so that carpus, elbow and shoulder are flexed and thereby provoked. After holding this position for about 30-60 seconds, the horse is released and then immediately trotted in a straight line about 20 meters away from the veterinarian. The horse is then turned around and trotted the same way back to the veterinarian. During the trot the veterinarian estimates the degree of lameness.

Description of proximal flexion test back leg: The veterinarian stands beside the horse and grabs the metatarsus of the leg and pushes it upwards so that hock, knee and hip are flexed and thereby provoked. This step is also performed for 30-60 seconds and the remainder of the test is as described above.

Description of distal flexion test front and back leg: The veterinarian stands by the cranial aspect of the leg. The hoof is lifted and the toe of the hoof is grabbed and the veterinarian pulls the toe towards him-/herself so that the three distal phalanges are flexed and thereby provoked. The remainder of the test is as described above.

Medical records

The medical records containing all information about the description of the horse (age, breed, sex etc.), medical history, examination, evaluation, diagnosis and treatment were collected from the medical record system at Löberöds horse clinic. The data was then compiled into an Excel file and analyzed using Excel.

The variables age, breed and sex were available for all horses and could easily be collected from the medical records.

The medical history, in those cases where noted, was limited to what was earlier diagnosed at Löberöds horse clinic, and could therefore be collected from the medical records. However, the duration of the lameness was in many cases unknown because the owners did not know. In the cases where the owners knew for how long their horse had been lame, this information was noted.

When it came to the lameness examinations not all horses were initially lame. For those that were initially lame, some were only lame on the lunge, but not in a straight line or vice versa. In the cases, where the horses were initially lame, the lame leg was noted as initial lameness regardless if the lameness was noted on a straight line or on the lunge. The flexion tests with the most and least intense flexion test reactions were observed, collected and used as source material.

The last piece of information that was collected from the medical records was the diagnosis. In some cases, no diagnosis was concluded. This was often due to the owners or the veterinarians choosing to close the case prior to a definite diagnosis. In some of these cases the lameness was irrelevant for the usage of the horse and in other cases there were other medical problems that were of greater importance than the lameness. In additional such cases, the veterinarians could not find the cause of the lameness.

Video recordings

All video recordings included in the study were performed by the author. The camera used was a pocket type digital camera (Canon Legria HF R78). The filming was done handheld. The aim was to stand at a distance of approximately 1.5-2 meters from the head of the horse. This distance varied, though, depending on where the horse stood in the running aisle during the flexion test. Another aim was to keep the camera at the same height as the horses' head and film the head slightly from the front so that eye, nostrils, ears and facial side muscles could be seen. The name of the horse and specific leg and joints that were flexed were spoken out loud into the camera microphone just before the shooting of the face started. Each film sequence lasted for 20-60 (most often about 40) seconds depending on the length of the flexion test.

Video editing

For each horse, three films were selected to be included in the study. The control film (where no flexion test was performed), one film where no or very little flexion test reaction was noted and one film where the most intense flexion test reaction was observed. Each sequence was cut down to between 15 and 50 seconds depending on the length of the flexion test and the quality of the film. Since most films already were about 20-60 seconds of length, the only sequences that were edited out were the ones where you could not see the face of the horse clearly. The editing was performed by the author (not blinded) and the software used was Camtasia studio 8 ® software.

The film sequences were put into two different power point presentations in a randomized order. The randomization was carried out using a list randomizer at www.randomizer.org. The reason why the film sequences were put into two different power point presentations was that the medical records for some horses were finished later than the others. The medical records had to be finished in order to be able to pick out the right film sequences for scoring.

Observer blinded pain scoring

The power point presentations containing the randomized clips of horse faces were shown to two blinded observers. They are both veterinarians with extensive experience in evaluating pain faces of horses. The observers were asked to pain score the horses in the presentation using the pain face scale, produced by Gleerup and her researchers (Gleerup *et al.*, 2015). They were allowed to play the sequences as many times as they needed or wanted and were then asked to make a consensus-agreement on whether the horse showed no pain face (score of 0), pain face present (score of 1) or intense pain face (score of 2). The result was subsequently presented to the author in an excel-sheet. The author then linked each individual score from the list of randomized results to the individual score from the list of randomized results to the individual score to plot it accordingly into the same excel-sheet as the data collected from the medical records.

Statistical analysis

To be able to describe the source material in further detail, descriptive statistics in the shape of pivot-tables and diagrams were used with the help of Excel. The descriptive statistics involved distribution of age, gender, breed etc.

Percentage-calculation was initially performed for a general overview description of the study results.

To investigate whether the result of the study was statistically significant, Fisher's exact test was performed. Since the horses acted as their own control in this study, the groups were related. The significance level was chosen to be $p<0,05$.

RESULTS

Source material

The study included a total of 24 horses. The distribution of age, gender and breeds are illustrated in figures 2-4 below. The age of the horses ranged from 4 - 17 years and the median age was 13 years. The age of the horses was not normally distributed. Out of all the included horses, 54% were geldings, 42% were mares and only 4% (one horse) was a stallion. When it comes to breeds, 50% of the horses in the study were Swedish warmblood. The remaining 50% of the horses were distributed over 9 other breeds.

Figure 2. *Age.*

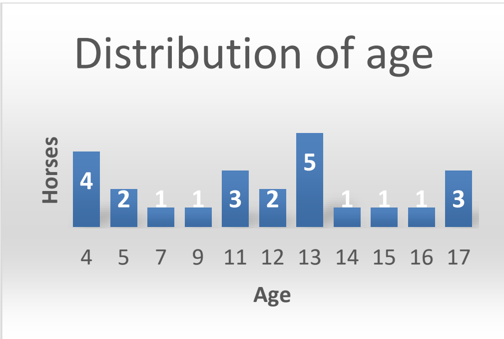


Figure 3. *Gender.*

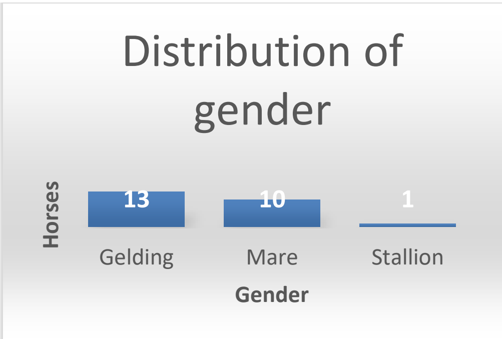
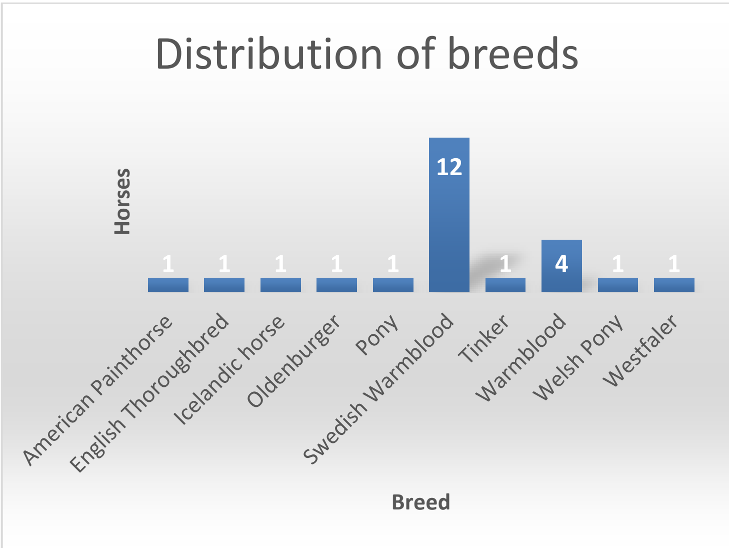
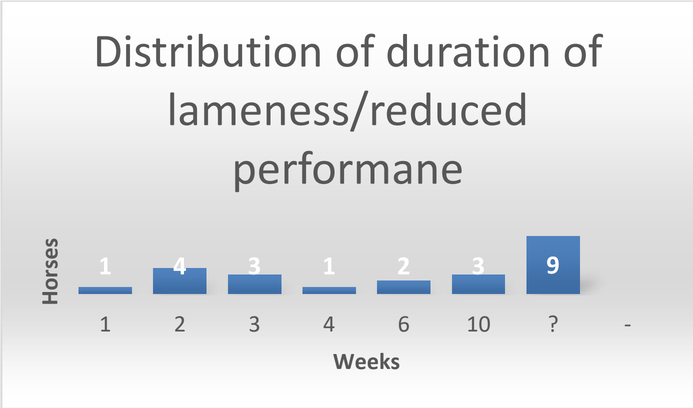


Figure 4. *Breeds.*



The known duration of lameness/reduced performance varied between one until ten weeks (distribution illustrated in figure 5 below). All horses that had been constantly lame for the last 12 weeks were excluded since these were defined as chronic pain patients, with the expectation of them showing signs of chronic pain, including a pain face. However, nine of the 24 horse owners did not know/remember for how long their horses had been lame or for how long they had experienced reduced performance.

Figure 5. *Duration of lameness/reduced performance.*



The inclusion criteria did not require the horses to show initial lameness. However, about 2/3rd of the horses showed some extent of initial lameness. The inclusion criteria did require all horses to have at least one positive flexion test reaction. The distribution of the degree of initial lameness and lameness following the flexion tests are illustrated in figures 6 and 7 below.

Figure 6. *Initial lameness.*

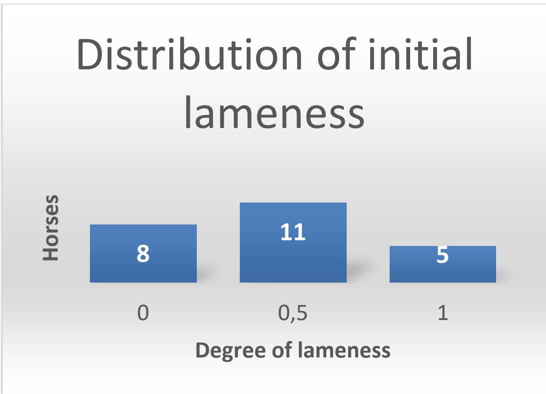
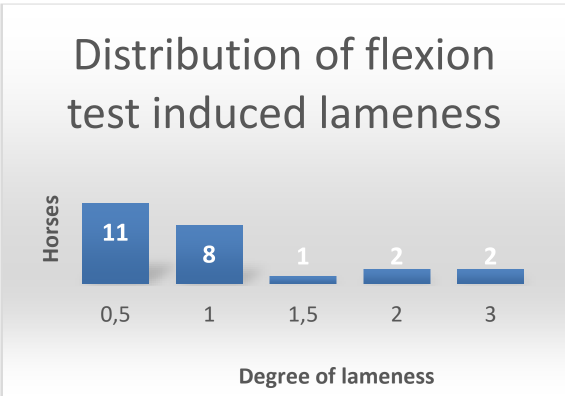


Figure 7. *Flexion test induced lameness.*



In 1/3rd of the cases no diagnosis was reached. The reason for this was that in some cases the lameness was considered so slight that it was negligible considering the usage of the horse. In other cases, there were other problems with the horse which needed to be concluded prior to the lameness. In some cases, further examinations needed to be done to be able to find the right diagnosis. In about 1/3rd of the cases the diagnosis was joint associated. The distribution of joint diagnoses is further illustrated in figure 9. In the remaining 1/3rd the diagnoses varied, see figure 8.

Figure 8. *Diagnoses.*

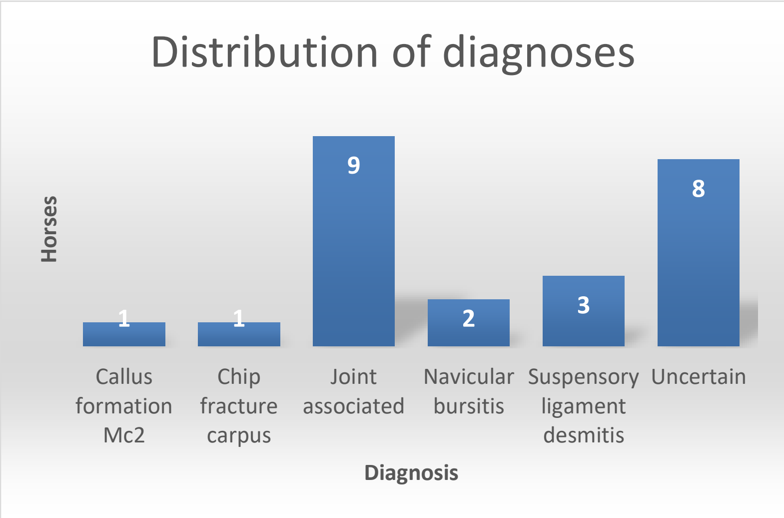
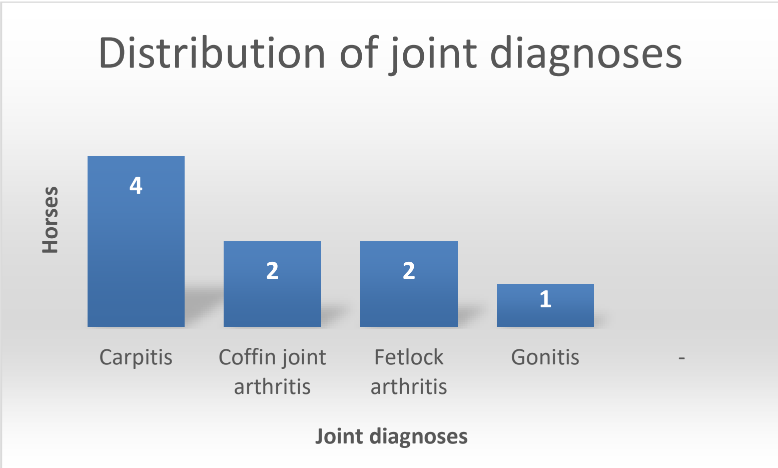


Figure 9. *Joint diagnoses.*



Facial expression of pain

The results from the pain face scoring are illustrated in table 1 below.

Table 1. *Results from pain face scoring.*

	PF lame leg	PF non-lame leg	PF control	Diagnosis	Duration of lameness (weeks)
Horse 1	1	1	0	Uncertain	-
Horse 2	0	0	0	Fetlock arthritis	-
Horse 3	1	0	2	Carpitis	6
Horse 4	1	1	1	Gonitis	-
Horse 5	0	1	0	Navicular bursitis	1
Horse 6	0	0	1	Carpitis	-
Horse 7	0	1	1	Uncertain	-
Horse 8	1	0	1	Suspensory ligament desmitis	10

Horse 9	1	0	0	Fetlock arthritis	3
Horse 10	1	0	0	Navicular bursitis	2
Horse 11	0	0	0	Coffin joint arthritis	2
Horse 12	0	1	0	Uncertain	-
Horse 13	1	0	0	Uncertain	-
Horse 14	0	0	0	Callus formation Mc2	-
Horse 15	2	1	1	Uncertain	-
Horse 16	1	1	1	Suspensory ligament desmitis	2
Horse 17	1	0	1	Chip fracture carpus	3
Horse 18	0	1	0	Coffin joint arthritis	2
Horse 19	1	1	0	Carpitis	10
Horse 20	0	1	1	Suspensory ligament desmitis	10
Horse 21	1	2	0	Uncertain	-
Horse 22	0	0	0	Carpitis	6
Horse 23	1	0	0	Uncertain	3
Horse 24	1	1	0	Uncertain	4

To test the hypothesis that horses with positive flexion test reaction show pain face while horses with negative flexion test reaction do not, a Fisher exact test with two-tailed p-value was performed. The significance level was chosen to be $p<0,05$. Two 2x2-tables were designed. One was designed to test whether there was any significant difference in the facial expression of pain between the groups "lame+" (lame) and "lame-" (non-lame). The other table was designed to test whether there was any significant difference in facial expression of pain between the groups "lame+" (lame) and "control" (no provocation). The tables are presented below in table 2 and 3. No significant difference could be seen in the presence of a facial expression of pain in neither of the cases (p-value 0,7725 respective 0,2476).

Table 2. *Pain face related to positive flexion test reactions versus negative flexion test reactions.*

	PF +	**PF -**	Total
Lame +	14	10	**24**
Lame -	12	12	**24**
Total	**26**	**22**	**48**

p = 0,7725

Table 3. *Pain face related to positive flexion test reactions versus control sequences.*

	PF +	**PF -**	Total
Lame +	14	10	**24**
Control	9	15	**24**
Total	**23**	**25**	**48**

p = 0,2476

To further analyze the result from the study some simple calculations and findings were done, presented below.

Five of the horses showed pain face (score of 1) while the non-lame leg was flexed, while they showed no pain face (score of 0) during flexion of the lame leg. One additional horse showed a higher degree of pain face (score of 2) when the non-lame leg was flexed compared to when the lame leg was flexed (score of 1). This means that six of the 24 horses showed facial expression of pain to a higher extent when a non-lame leg was flexed compared to a lame leg.

Two of the 24 horses showed the same degree of pain face (score of 1) throughout all of their scored film sequences. The horses thus showed facial expression of pain irrespective of whether they were provoked by flexion tests or not. Neither was there any difference in the scoring of pain face between positive or negative flexion test.

One of the horses only showed pain face when there was no flexion test (provocation) performed at all. The score for this sequence was 1. The scores for both positive and negative flexion tests for this horse were 0.

Three of the horses never showed a pain face in any of the scored film sequences. These three horses were all diagnosed with different diagnoses. One of them had a fetlock arthritis, another had a carpitis and the last one had a callus formation on Mc2.

About 58% of the horses showed some degree of pain face during flexion of the lame limb, while the other 42% of the horses showed no pain face during flexion of the lame limb.

Fifty percent of the horses showed some degree of pain face during flexion of the non-lame limb whereas the other fifty percent showed no pain face in the same situation.

About 38% of the horses showed a pain face when no leg was flexed. Out of these horses, 44% had some previous history of lameness.

Fifty percent of the horses which had a previous history of lameness showed a pain face when there was no flexion test performed.

DISCUSSION

Pain face

In this study horses with both positive and negative flexion test showed facial expressions of pain. Also some horses that were left without provocation showed pain face. The hypothesis that horses with positive flexion test had more facial expressions of pain than horses with negative flexion tests or horses that were not provoked was thus unsubstantiated. The reasons for this may be many.

A possible cause may be that the horses react so individually to the environment that the results from the pain face scoring cannot be compared across horses. Some horses had been to the clinic several times and may therefore have felt more relaxed, or stressed, than other horses in the same situation. The clinical circumstances were also different for the different horses and for different flexion tests. Such differing circumstances include possible neighing from the stable, a clipper in the treatment room, a door banging from the office, etc. All of these unforeseen occurrences that are hard to control for might have caused changes in the mood of the horses. These mood changes could possibly have led to an intensifying or reducing of some facial expressions. Individual variations should be taken into consideration when, for example, looking at the result for the horses that never showed any pain face. Perhaps these horses are less sensitive for pain than the other horses.

Another possible cause for the results might be that the equine pain face scale might not be applicable to horses with orthopedic conditions. Maybe the pain induced by tourniquet or capsaicin (Gleerup *et al.*, 2015) or post-operative pain related to castrations (Dalla Costa *et al.*, 2016) is perceived very differently by the horse compared to the orthopedic pain that is induced by flexion tests, thereby possibly resulting in different facial features.

An important aspect is that this study only included 24 horses. That is a rather small source material, considering that the diagnoses, ages and breeds of the horses were very variable. Perhaps if the study was performed with a greater amount of horses of the same age, breed, gender etc., the results would be different. In this study all horses that showed a minimum of 0,5 degrees' lameness after flexion test were included. Perhaps there would be a larger consistency in the result if the inclusion criteria were narrower and only included horses with a higher degree of lameness after flexion test.

It may also be speculated whether some of the horses included in the study suffered from pain that was present constantly, for example chronic pain. This could be associated with exhibition of a pain face more or less constantly, regardless if a painful joint was flexed or not. One can also postulate that the pain from standing on the lame limb while another is being flexed - and thereby putting more weight than usual on the limb - could be equivalent to exposing that lame limb to a flexion test. The chronic pain is a complicated factor to evaluate in this study, since we do not have the complete history of every single horse and since we only have very short momentarily shots of the horses. One possible way to try to weigh in the chronic factor in the study would be to film the horses in a normal environment for the horse (for example a box in their own stable) for a longer period of time. This could for example have been done with a hidden camera during the day or night before the visit to the clinic. Chronic pain face and/or pain behavior could then have been evaluated outgoing from this. The reason why this wasn't performed was depending on practical issues and time limitation. The horses visiting the clinic come from a large area (Skåne, Blekinge, and southern Småland). This would mean that it would be a very time consuming job to drive around to all the stables and put up the cameras. It would also require a whole lot of cameras, since some days up to eight horses were filmed in one single day (this would mean that eight horses had to be filmed in their own stables the night before the visit). Another suggestion could be to film the horses in the boxes of the clinic stable before the lameness evaluation. This was unfortunately not possible, though, since many of the horses did not stand more than a couple of minutes in the boxes, and some did not stand in the boxes at all.

The fact that the diagnoses were very variable (see figure 8, figure 9 and table 1) might also contribute to the inconsistency in the result. One theory might be that different types of pain gives rise to slight alterations in the pain face of the horse. If this was the case there might be differences, for example, depending on if the pain originates mainly from bone tissue, soft tissue, joint capsule etc. or if it is mainly inflammatory or not. When looking at the diagnoses in this study, one realizes that the pain originates from several different types of tissue in the different cases and the pain is in some cases assumed to be inflammatory and in some cases not.

Something that should not be forgotten in this study is that lameness evaluation was performed as a subjective estimation. The human factor should not be underestimated. Since the horse practitioners in this study have extensive experience in lameness diagnostics this should not be a large problem, but to make it more objective we could, for example, have used some sort of objective tool to measure the lameness, for example a lameness locator. Something that should also be taken into consideration is that the flexion test result maybe isn't always completely in relation to the pain that is experienced by the horse during the flexion. It might be that some diagnoses give rise to more pain during flexion than when trotting afterwards or vice versa.

One interesting detail is that all horses with suspensory ligament desmitis (three horses) - see table 1 - show some degree of pain face when not provoked at all. Since it's only three horses, this might of course be a coincidence, but one can also imagine that this is a diagnosis that gives rise to pain when standing still on all four legs. This is something to also take into consideration when looking at the horses with joint pain. These are thought to be painful both at flexion and at loading, which could mean that some horses get a "false positive" pain face when the contralateral limb is flexed.

Limitations of study

All horses included in the study were horses that came to one specific clinic for an examination during the month of September. Due to this, the distribution of area of use and breed might not be representative for the whole Swedish horse population. It could be that horse owners with horses used for cross country or jumping prefer to come to this specific clinic. If this was the case, there could be a subsequent uneven distribution. The fact that the filming was performed during one particular month could mean that variations depending on season cannot be adjusted for. One could hypothesize that some diagnoses are more common in the autumn than in the spring (different times in competition season etc.) and this is not taken into account in this study. However, since only 24 horses are included in the study, it would be impossible to get a representative selection of horses, diagnoses etc. that would correspond to the Swedish horse population.

In contrast to the studies by Dalla Costa (2016) and Gleerup (2015) this study was performed in less controlled conditions. There are several factors that might have influenced examination, filming, behavior of the horse and evaluation of the films. The examination was performed by two different clinicians with extensive experience in the field of lameness examinations. The clinicians did not receive any specific instructions in how to perform the lameness examinations (including flexion tests) but were asked to work as they usually do. This means that neither time nor force are standardized in the flexion tests. Some horses are only flexed for 20 seconds while some are flexed for more than 60 seconds. This is worthy of reflection since many publications discuss the importance of standardizing force and time (Verschooten & Veerbeck, 1997; Keg *et al.*, 1997; Armentrout *et al.*, 2012).

The quality of the filming was partly dependent on how the horse reacted and behaved during the filming. Some horses stood very quietly and still. In these cases, it was easy to catch film sequences where the facial expression could easily be evaluated. However, other horses tried to get loose, shook their heads, jumped back and forth etc. In these cases, the filming was harder to perform in a satisfactory way. Some horses frequently interacted with their owners during the film sequence, making the evaluation harder. Another factor which made some films hard to evaluate was that there was a window in the running aisle, which made the faces of the horses look very dark when filming with the window as a background. Despite efforts trying to avoid this window it still became impossible in some cases.

The behavior of the horse was probably dependent on several different factors: pain level, how much handled the horse was, the age and temper of the horse and other environmental influencing factors in the clinic at the moment. The latter was exemplified by horses neighing in the stable, cars driving at the parking space outside etc. These were also factors that might have affected the facial expression of the horses and the possibility to evaluate the film sequences. Nevertheless, all of these factors are factors that are more or less always present in a clinical environment.

The evaluation of the film sequences was performed by two veterinarians with extensive experience in evaluating facial expression of pain in horses. The result was presented as a consensus-agreement, which means that the observers discussed with each other before deciding the final score for each film sequence. Since it is humans who visually evaluate the facial expression of the horses, this part of the study is impossible to make totally objective. To be able to make this part objective the film sequences would, for example, have to be presented to a computer program designed to evaluate facial expression of pain in horses. No such program exists as of the time of this writing.

CONCLUSION

No significant difference in the grading or presence of pain face could be seen when comparing positive versus negative flexion tests. The same was the case for comparing positive flexion tests versus control sequences.

Recommendations for further studies

One recommendation is that such a future study could be done with more specified inclusion criteria and even more standardized situations. Such inclusion criteria could include horses with a degree of lameness higher than for example 2 degrees following a flexion test. Standardized situations could, for example, be designed by using an environment where no disturbing noise caused by clinical circumstances (such as neighing, cars, clipping machines etc.) could arise. I would also recommend not to ask the horse owners to hold their horses during the flexion tests, but to use one independent person who holds all the horses. This person should aim not to interact with the horses at all during the flexion tests.

Another recommendation is to further expand the observation parameters of the faces of the horses with orthopedic pain induced by flexion tests. One could try to quantify additional behaviors/face expressions such as licking/chewing, lowering of head, turning their head towards the flexed leg, eye blinking etc. Perhaps some behaviors/face expressions are more linked to orthopedic pain compared to other types of pain and vice versa.

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