Universiteit Utrecht

Faculty of Veterinary Medicine

Master Thesis

Assessment of acute pain in donkeys using the Donkey Composite Pain Scale (Do-CPS)

J.A.M. Smolenaers
5487366

Mei 2020

Supervisors:
J.P.A.M. van Loon
M.C. van Dierendonck

Table of contents

Abstract

Although there is shared heritage between the donkey and horse, they are remarkably different in their behaviour and physiological traits. Therefore, it is no longer acceptable to simply consider a donkey as a small horse and do donkeys need their own species-specific validated tools for the assessment of pain.

In a composite pain scale (CPS), several putative pain-related parameters are evaluated concomitantly and are scored individually. This study investigates the clinical applicability, the validity and inter-observer variability for a refined CPS designed for donkeys; the Donkey Composite Pain Scale (Do-CPS).

A total of 159 donkeys (n = 44 with acute pain, n = 115 healthy pain free controls) have been assessed by direct observations, using the Do-CPS. The patients were all suffering from acute pain caused by different medical conditions (lameness, facial pain, post-operative pain and colic) and were observed for at least two to three days. The Do-CPS scores showed high inter-observer reliability ($R^2 = 0.95$, $P < 0.001$). The cut-off value for differentiation between healthy pain free and acute pain donkeys was 5. The Do-CPS showed statistical significant differences between patients and control donkeys for all patient subgroups.

Sensitivity and specificity were good (sensitivity 73%, specificity 100%), in particular for the subgroup ‘lameness’ (sensitivity 92%, specificity 100%). After applying weighting factors to the individual parameters, sensitivity improved however specificity reduced for all patient subgroups. The use of the Do-CPS enabled repeated and objective scoring of pain in donkeys with acute pain, especially for the patient subgroups ‘lameness’ and ‘colic’.

Introduction

Zimmermann defined pain in animals as: “*an aversive sensory experience that elicits protective motor actions, results in learned avoidance and may modify species-specific traits of behavior including social behavior*”.[1] As pain is a subjective experience that cannot be verbally communicated by animals, recognizing signs of pain in animals is very important but at the same time also difficult. Physiological parameters, such as heart rate and respiratory rate may be affected by pain and are easily measured and quantified. However, these parameters are not specific for pain.[2,3] Not only physiological parameters change when animals are experiencing pain, also changes in behavior can be seen. The subtle or overt changes in behavior offer an indication of the presence, localization and severity of the pain.[4]

Although there is shared heritage between the donkey and horse, they are remarkably different in their behavior and physiological traits. For instance, the donkey has different temperature, pulse and respiration ranges compared to horses. In addition, the fight instinct of the donkey is more easily engaged than that of the horse, whose default reaction is nearly always flight.

Since the donkey is a unique species of the Equidae family, it has considerable specific differences in comparison to the horse; such as pain recognition.[5] Donkeys seem to more effectively mask signs of chronic or low to moderate grade pain than horses do. For example, when a horse is suffering from colic it can show signs of rolling, flank watching and kicking, compared to a donkey which very often only shows moderate signs, such as a lowered head position, backwards/sideways pointing ears and isolating themselves from the group.[6]

Systematic assessment of pain, using defined and validated pain scoring scales, will help to improve recognition and treatment of painful conditions in donkeys. In previous reviews about pain assessment in horses[4,7], different scales that could be used for pain assessment in horses were described. For instance, the visual analogue scale (VAS), simple descriptive scale (SDS), numerical rating scale (NRS), facial expression pain scale (FEPS) and the composite pain scale (CPS).

The VAS is a simple one-dimensional pain scale, it consists of a 10 cm horizontal line, representing pain intensity that starts on the left with 0 (no pain) and ends at the right with 10 (the worst imaginable pain). The observer places a mark on this line that corresponds to the perceived amount of pain an animal is experiencing. The pain score can then be read off as the number of millimeters from the zero end of the line. The VAS is very easy to use. However, because of a poor inter-observer reliability, the VAS scale has proven to be unreliable for pain evaluation in horses.[8–10]

In the current study a composite pain scale (CPS) is used, because pain is a complex phenomenon evoking emotional, behavioral and physiological responses. In a CPS, these several putative pain-related parameters are evaluated concomitantly and are scored individually using well-defined classes by means of several simple descriptive scales covering these different elements that are related to pain expression. Each SDS consists of pre-defined classes of pain to which an index number is assigned to allow the data to be handled statistically. For example; 0, no pain; 1, mild pain; 2, moderate pain; 3, severe pain. These index numbers are then combined to provide an overall CPS score. Therefore, the CPS can better identify and quantify pain than one of the other pain assessment systems.[4]

Several studies have been published in relation to pain assessment in horses using a CPS type scoring system.[10–14] Originally, the CPS of Bussières et al.[12] was developed for horses with acute orthopaedic pain. However, the study of van Loon et al.[11] showed that this CPS contains various parameters that can also be applied for visceral pain in horses as well. A few years later, the Equine Utrecht University Scale for Composite Pain Assessment (EQUUS-COMPASS) was developed for the assessment of acute equine visceral pain, which has been described in studies of van Loon and van Dierendonck[10,15].

Due to their fundamentally different biology and behaviour it is essential that donkeys have their own species-specific validated tools for the assessment of pain. In 2017, Daja van Nunen developed in a study for her master thesis a FEPS for donkeys (unpublished data). A few months later, Simone Gertzen and Tess van Overbeek went to 'The Donkey Sanctuary' in Sidmouth (England) to conduct two studies for their master theses (unpublished data). Gertzen conducted a study about testing the FEPS for donkeys and van Overbeek conducted a study about the development of a CPS for donkeys.

The CPS used in the study of van Overbeek (unpublished data) is refined, based on the outcome of the study, and resulted in the Donkey Composite Pain Scale (Do-CPS) that was used in the current study. Furthermore, the data of the study of van Overbeek (unpublished data) will be included in this study.

The aims of this study are to further refine the Do-CPS, and to investigate the clinical applicability, the validity and inter-observer variability of the Do-CPS in different types of acute pain in donkeys. This pain scale should be useful for clinicians, researchers and handlers of donkeys. It would allow them to recognize and quantify pain more easily when donkeys are coping with potential painful conditions. This would be beneficial for donkey welfare.

The hypothesis is that the Do-CPS, used in this study, is able to differentiate between patients and healthy pain free control donkeys, has a good inter-observer reliability and can therefore be used for pain assessment in donkeys suffering from different types of acute pain.

Material and methods

Animal selection

For this study, twenty-nine donkeys with an acute painful condition that were reported to the Veterinary Department of 'the Donkey Sanctuary' in Honiton (England) between the 16th of October 2017 and the 14th of November 2017 have been observed. The donkeys were collected from six different farms that are part of 'The Donkey Sanctuary' in Sidmouth (England). The patients were listed categorized by their specific medical conditions: lameness, facial pain, post-operative pain or colic. In this group of patients, the pain score has been used for documentary purposes only, and clinical decisions about the initiation of analgesic or other treatments were at the discretion of the attending veterinarian and independent of the Do-CPS scores. If available, the final diagnose and treatment plan were recorded after observation.

For each patient two to three control donkeys were observed. The social partner (if present), a similar (i.e., age, gender, weight, size) donkey in the same group and a similar donkey from another group. The control donkeys were only included when they were free from clinical problems and did not receive any analgesic treatment. In total seventy-eight control donkeys were observed.

Donkeys that appeared to have no painful condition are not useful for this this study, thus these were excluded from the dataset retrospectively (n = 12).

To obtain a larger dataset, the results derived during the study of van Overbeek (unpublished data) were included in this study (19 patients and 49 control donkeys). Table 1 and Appendix 3 show a list of characteristics and the medical condition of all patients used in the current study.

Table 1 *Animals used in this study.*

	Patients	**Controls**
Age (years)		
Mean (±SD*)	16.8 (9.0)	16.5 (8.7)
Min-Max	1 - 37	0 – 35
Gender		
Jack (stallion)	3	1
Gelding	27	72
Jenny (mare)	14	42
Condition		
Lameness	24	66
Colic	7	18
Facial pain	7	17
Post-operative pain	6	14
Total number	**44**	**115**

* SD = standard deviation.

The Donkey Composite Pain Scale (Do-CPS)

The Do-CPS (Appendix 1) is a multifactorial SDS based on several parameters that are thought to be specific for pain. It includes physiological parameters, responses to stimuli, and spontaneous behavioral parameters. Each of the parameters is scored from 0 to 3, leading to a total summed Do-CPS score: ranging from 0 (no signs of pain) to 60 (maximal signs of pain).

The Do-CPS that has been used in this study is based on the CPS for donkeys (Appendix 2) which van Overbeek (unpublished data) used in a previous study about the development of the CPS for donkeys. This last CPS is, in turn, based on the EQUUS-COMPASS, which has been described in studies of van Loon and van Dierendonck[10,15] and on the CPS by Bussières et al.[12] These composite pain scales were developed for assessment of acute equine visceral and orthopedic pain in horses. The suggested improvements for the CPS for donkeys, that resulted from the previous study of van Overbeek (unpublished data), were processed into the Do-CPS.

The first suggested improvement involved the parameter ‘Head carriage’. The normal head carriage of donkeys is lower in comparison to horses, which makes it more difficult to define a ‘Lower carriage of the head’. Furthermore, a clearer explanation has been given about the normal position of the head, which is in general a position of the head above the withers, and when donkeys are eating out of a trough or grazing, the head is below the withers but should still be considered as a normal position.

Secondly, the scoring possibilities of the parameter ‘Reaction to palpation’ were not distinctive enough. For this reason, the parameter has three scoring possibilities in the Do-CPS, like the parameter ‘Reaction to observer(s)’.

The original parameter ‘Position of the ears’ included three scoring possibilities, it was found that it is difficult to distinguish these three possibilities. Therefore, the Do-CPS includes only two scoring possibilities which are ‘Normal position’ or ‘Abnormal position (backwards/sideways/flat)’. Since it could take a while for a donkey to start showing signs of pain, especially after being approached, the position in which the ears are for more than 75% of the time is scored.

The parameter ‘Movement’ needed more explanation regarding the severity of the lameness to improve objectivity. Therefore, the scoring option ‘No reluctance to move and/or mildly abnormal gait’ has been changed into ‘Mildly abnormal gait (1 or 2 out of 5 lameness) and/or stiff walk’ and the scoring option ‘Reluctance to move when motivated and/or severely abnormal gait’ has been changed into ‘Reluctance to move when motivated and/or severely abnormal gait (3 to 5 out of 5 lameness)’.

In the previous study of van Overbeek (unpublished data) the parameter ‘Sweating’ was only scored once: this individual sick donkey was suffering from severe colic and was damp to touch and therefore a score of 1 was given with the first version of the CPS for donkeys. Since donkeys with colic rarely sweat[16], any signs of sweating should be given a higher score in the Do-CPS. Therefore, ‘Signs of sweating (wet spots visible, no droplets or streams)’ will now result in a score of 2 in the Do-CPS.

Moreover, in the study of van Overbeek (unpublished data) rolling was only seen in healthy pain free donkeys for auto grooming. To prevent giving an auto grooming control donkey a score of 3 in the Do-CPS for rolling, the parameter is now described as ‘Lying down, rolling (excluding auto grooming)’.

Furthermore, several adjustments have been made to make the Do-CPS more specific and easier to use. For example, for the parameter ‘Eating’ the scoring possibility ‘Hesitates to eat’ is removed from the Do-CPS and the scoring possibility ‘Eats less and/or eats slowly’ is added instead of ‘Eats less or pretends to eat’.

Lastly, the order of the parameters was changed; the parameters whereby physical interaction with the donkey is necessary were moved to the end of the Do-CPS.

The total CPS scores, derived during the study of van Overbeek (unpublished data), have been adjusted to the improvements of the Do-CPS as mentioned above. Therefore, the dataset of the study of van Overbeek (unpublished data) could be used in this study.

Experimental design

Three pain scales were used to collect data: the Do-CPS, the FEPS and the VAS. The FEPS is a pain scale that has been developed and used in a previous study period and is now tested with similar aims by Annechien Smalbroek.

Observations for the Do-CPS and FEPS were performed by two observers (veterinary master students) who performed their observations pairwise, simultaneously but independently: during the pain assessment, the observers did not discuss their findings. The observers were not blinded for the reason the veterinarian was visiting and they were aware of the treatment plan. Furthermore, the observers were, as long as possible, standing a few meters away from the donkey to not disturb the donkey.

The observers were given the chance to familiarize and train themselves with the parameters in the Do-CPS and FEPS using donkeys of ‘Stichting De Ezelsociëteit’ in Zeist (The Netherlands) before the beginning of the study. These donkeys were not included in the analyzed dataset.

Patients were evaluated as soon as possible after the observers arrived at the barn and preferably before administration of any analgesics. A few times it was not possible to obtain the pain scores before the donkey was moved to the clinic; the veterinarian had started the clinical examination or the veterinarian had already administered analgesic medication (or the patient was on long term analgesic medication). These patients were included in the study after making a note of the situation and, if necessary, the analgesic medication.

A fixed order of observation was used: at first, the FEPS was assessed and then the Do-CPS (ending with the physiological parameters) was assessed. After the observation, a video recording of the donkey was made. Hereby it was possible to observe the donkey again at any time if the observers wanted to. However, the first assessed FEPS and Do-CPS scores were used. In this video, at first the whole body of the donkey was filmed, then the head of the donkey was filmed and at last the donkey was filmed while walking. Lastly, the VAS score was received from the treating veterinarian (whenever the veterinarian was available) to make sure this score would not interfere with our own findings.

In addition to the collected behavioral data, the clinical data of the patients and their controls were collected from the database of ‘The Donkey Sanctuary’ (Microsoft Dynamics CRM). Information such as age, current medical status, current medication and other facts that might be important were registered in an Excel spreadsheet.

The patients were followed, if possible, for at least two to three days. The assessment of the FEPS lasted approximately two minutes and for the Do-CPS approximately eight minutes, so the total observation period lasted approximately 10 minutes. The donkeys were evaluated in their own barn with their group or in a stable at the hospital with their friend, preferably at the same time of the day.

Data processing and statistical analysis

The data of the patients consists of the mean score of the two observers at the first assessment of acute pain (T = 0). All data were collected in an Excel spreadsheet. Because of the ordinal nature of the data, data are expressed as median, quartiles and ranges and non-parametric tests were used to perform statistical analysis. The Spearmann correlation coefficient was calculated to assess the inter-observer reliability. The Mann-Whitney U test was used to compare patients with the controls and to compare specific controls (i.e., similar age, gender, weight, size) with the other controls. The patients were categorized in different subgroups, which were lameness, colic, facial pain and post-operative pain. Within these groups the patients were compared to their specific control donkeys, again by using Mann-Whitney U tests. Boxplots were made for the Do-CPS scores over time for the different subgroups of pain. The cut-off value of ≥ 5 for the Do-CPS was determined based on the data of the study of van Overbeek (unpublished data) to obtain maximal differentiation between patients and healthy pain free control donkeys. Sensitivity, specificity, and positive and negative predictive values were determined for the Do-CPS using this cut-off value. Sensitivity and specificity for individual parameters of the Do-CPS were determined using the data of van Overbeek (unpublished data). Based on these values, weighting factors for the individual parameters were determined retrospectively: when the percentage of sensitivity or specificity of the parameter was ≤ 25%, a weighting factor of 0; between 25% and 50%, a weighting factor of 1; between 50% and 25%, a weighting factor of 2; and when ≥ 75% a weighting factor of 3 was applied (Appendix 4). After using these weighting factors, sensitivity and specificity of Do-CPS scores were determined again and a cut-off value of ≥ 2 was used. Statistical analysis was performed with SPSS Statistics version 24.0 (IBM). Statistical significance was accepted at $P < 0.05$.

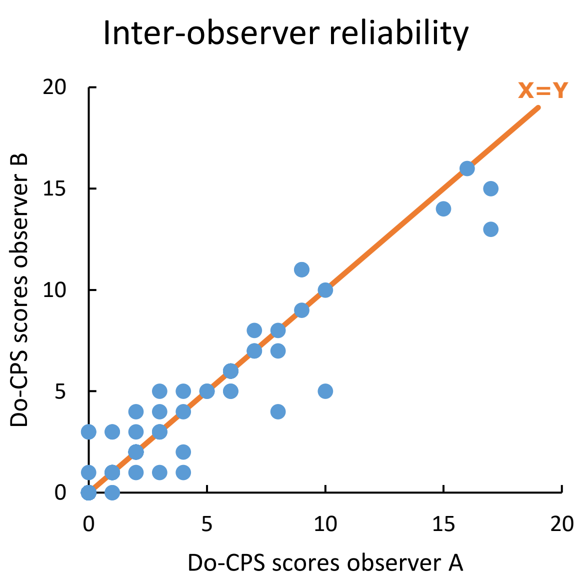

Fig. 1 *Scatter plot of Do-CPS scores (n = 130, R2 = 0.95, P < 0.001), assessed by two different observers at the same moment. The orange line in this figure shows the X=Y line.*

Results

Inter-observer reliability

Figure 1 shows the results of correlation analysis between the different pain scores of two independent observers. The Do-CPS showed strong and significant correlation ($R^2 = 0.95$, $p < 0.001$).

Differences between subgroups of control donkeys and patients

For the comparison between patients and healthy pain free controls, the mean pain score was taken as well as the mean control score. Figure 2 shows the comparison between patients and control donkeys, the Do-CPS scores of the patients are significantly higher compared to control donkeys ($P < 0.001$).

The Do-CPS scores showed no significant differences between specific control donkeys and other control donkeys for all of the patient subgroups ($P > 0.05$) (Fig. 3).

The same comparison was made for every subgroup of patients. The Do-CPS scores showed significant differences between all the subgroups of patients and their specific control donkeys ($P < 0.05$) (Fig. 3). In addition, the scores of the subgroups lameness and colic showed a stronger statistically significant difference ($P < 0.001$).

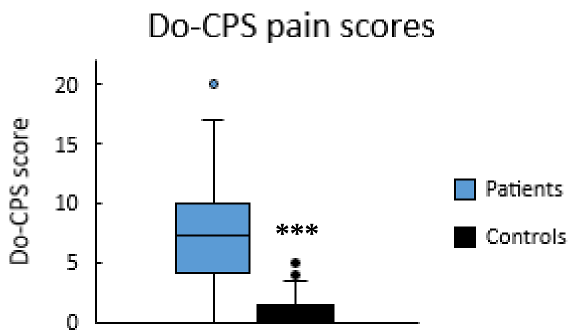

Fig. 2 *Mean Do-CPS scores of all patients (n = 44) versus all control (n = 115) donkeys. *** for P < 0.001 between patients and control donkeys. Lines in boxes show median scores; boxes show 25-75th percentiles; error bars show 5-95th percentiles.*

Effects over time for the different subgroups of patients

Figure 4 shows the Do-CPS scores over time for the different subgroups of patients. Because of differences in group size on different days, no statistical tests could be conducted on these data.

Sensitivity, specificity, positive and negative predictive value of unweighted Do-CPS scores

Table 2A shows sensitivity, specificity, positive and negative predictive values of Do-CPS scores using the cut-off value of ≥ 5 to differentiate between patients and healthy pain free controls.

Sensitivity and specificity of the weighted Do-CPS scores

Table 2B shows sensitivity and specificity for Do-CPS scores after applying the weighting factors of the individual parameters per subgroup of pain (Appendix 4). A cut-off value of ≥ 2 was used to differentiate between patients and healthy pain free controls.

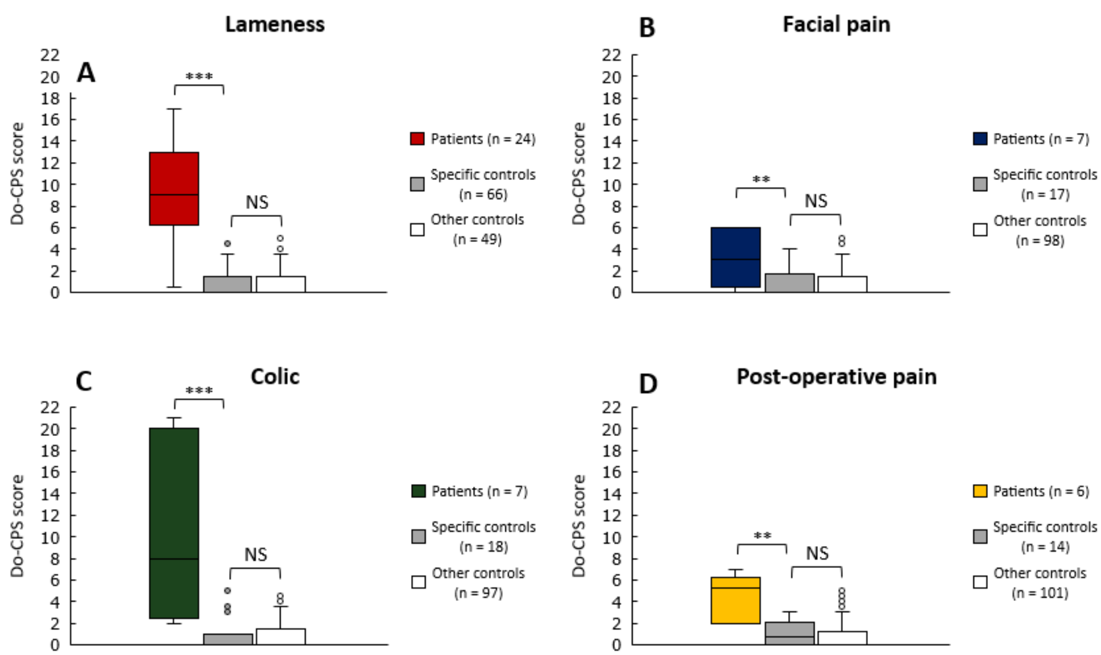

Fig 3. *Mean Do-CPS scores for lameness (n = 24) versus specific control donkeys (n = 66) and the other control donkeys (n = 49)(A), facial pain (n = 7) versus specific control donkeys (n = 17) and the other control donkeys (n = 98)(B), colic (n = 7) versus specific control donkeys (n = 18) and the other control donkeys (n = 97)(C) and post-operative pain (n = 6) versus specific control donkeys (n = 14) and the other control donkeys (n = 101)(D). *** for P < 0.001 and ** for P < 0.01 between patients and specific control donkeys. NS for no significant difference between specific controls and other controls. Lines in boxes show median scores; boxes show 25-75th percentiles; error bars show 5-95th percentiles.*

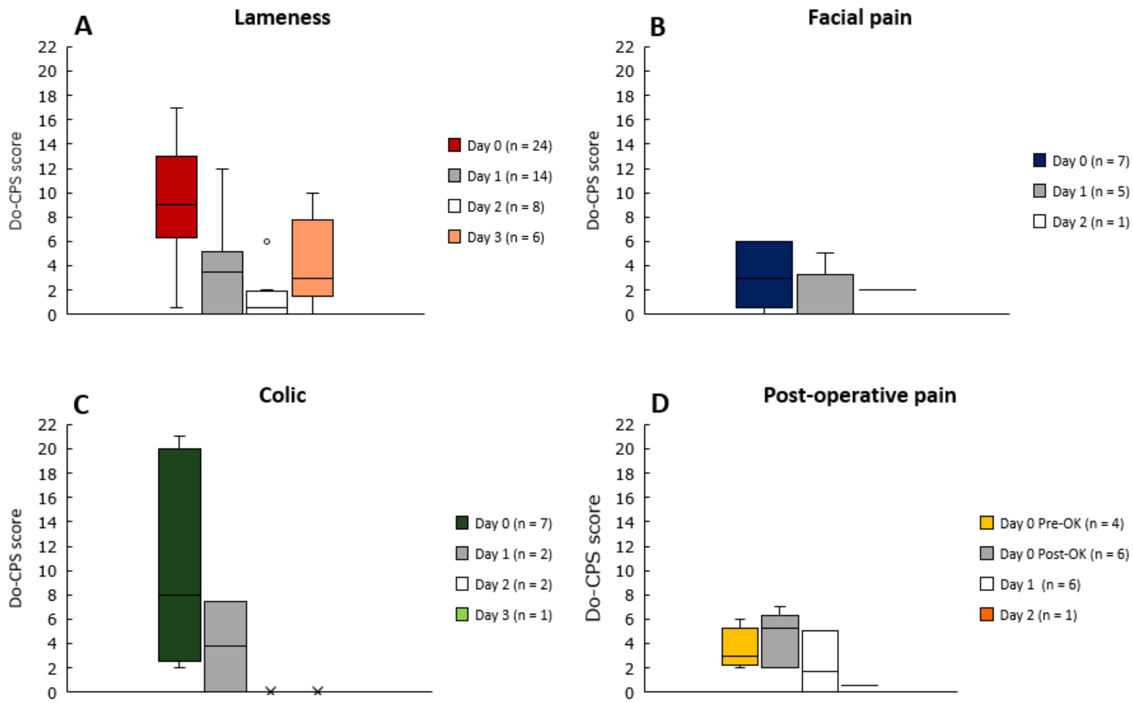

Fig. 4 *Mean Do-CPS scores over time for the patient groups lameness (n = 24) (A), facial pain (n = 7) (B), colic (n = 7) (C) and post-operative pain (n = 6) (D). Lines in boxes show median scores; boxes show 25-75th percentiles; error bars show 5-95th percentiles.*

Table 2 *Sensitivity, specificity, positive and negative predictive value of the unweighted Do-CPS values for the different subgroups of pain (A), and sensitivity and specificity of the weighted Do-CPS values for the different subgroups of pain (B).*

	Unweighted values[A]				**Weighted values[B]**	
	Sensitivity (%)	**Specificity (%)**	**Positive predictive value (%)**	**Negative predictive value (%)**	**Sensitivity (%)**	**Specificity (%)**
All data *Patients (n = 44), controls (n = 115)*	73	99	97	90	91	78
Patient group 'lameness' *Patients (n = 24), specific controls (n = 66)*	92	100	100	97	96	88
Patient group 'facial pain' *Patients (n = 7), specific controls (n = 17)*	29	100	100	76	71	88
Patient group 'colic' *Patients (n = 7), specific controls (n = 18)*	71	95	83	90	100	83
Patient group 'post-operative pain' *Patients (n = 6), specific controls (n = 14)*	50	100	100	82	100	64

[A] A cut-off value of ≥ 5 was used for differentiation between patients and healthy pain free control donkeys.
[B] A cut-off value of ≥ 2 was used for differentiation between patients and healthy pain free control donkeys.

Discussion

The Do-CPS showed strong significant difference between the patients and pain free control donkeys (Fig. 2). Therefore, the Do-CPS is able to assess acute pain in donkeys. After the patients were categorized by their specific medical conditions: lameness, facial pain, post-operative pain and colic, the same significant results were found (Fig. 3). Good sensitivity and specificity (unweighted values) for differentiation between donkeys with acute pain and healthy pain free control donkeys show that the Do-CPS is able to differentiate between different levels of pain (Table 2A). Sensitivity improved after applying weighting factors to the individual parameters, however, specificity was reduced for all patient subgroups (Table 2B). An inter-observer reliability correlation coefficient of 0.95 was found (Fig. 1).

The high inter-observer reliability of the Do-CPS scores ($R^2 = 0.95$) supported that the Do-CPS is an objective scale to assess pain in donkeys suffering from acute pain (Fig. 1). In the previous study of van Overbeek (unpublished data) a correlation coefficient of 0.79 was found. The increase of the inter-observer reliability shows that the suggestions for improvement, merged in the Do-CPS, were valuable to obtain a more objective scale. Furthermore, the high inter-observer reliability makes the Do-CPS suitable for clinical use by different observers.

In addition to a high reliability, it is important to note that the Do-CPS can distinguish between patients and healthy pain free control donkeys. The Do-CPS showed strong significant differences between all patients and all control donkeys (Fig. 2), which shows the Do-CPS's ability to differentiate between patients and pain free control donkeys. However, Robertson[17] argued that pain assessment systems for horses and donkeys must also consider different sources of pain. After the patients were categorized by their specific medical conditions: lameness, facial pain, post-operative pain and colic, the same significant results were found (Fig. 3). Donkeys in the subgroups colic and lameness showed higher pain scores, compared to their controls; while the donkeys in the subgroups facial and post-operative pain differed less strong from their controls. This indicates that the Do-CPS is more able to assess acute pain caused by orthopedic pain (lameness) and colic. The study of van Overbeek (unpublished data) found no significant results within subgroups of facial pain and post-operative pain. The presence of significant differences in the current study can be explained by the increased number of animals in the dataset. Therefore, a larger dataset is desired for further studies regarding assessing acute pain in donkeys with the Do-CPS, especially for the subgroups facial pain and post-operative pain.

The Do-CPS scores showed no significant differences between specific control donkeys and other control donkeys (Fig. 3). This refutes the assumption that finding a similar (i.e., age, gender, weight, size) control donkey for each patient is relevant.

A study of van Dijk et al.[18] showed that combining behavioral and physiological elements of pain in a CPS lead to higher validity, since it is well known that both behavioral and physiological pain parameters lack sensitivity and specificity when used individually. On the other hand, van Dijk et al[18] described that completion of a CPS is often time-consuming and this would not be beneficial for hospital cases with acute pain. However, after improving the descriptions for some of the scoring possibilities, the assessment of pain using the Do-CPS lasted approximately 5 to maximal 10 minutes. This corresponds with the study of van Loon and van Dierendock[15] were the observation period lasted 10 minutes.

The mean Do-CPS scores of the subgroup lameness show a sudden increase on day three (Fig. 4A), this was not due to a change in the analgesics program. These observations were conducted mostly early in the morning, therefore an explanation for the increased Do-CPS scores could be that pain assessment was done before the donkeys received their analgesic medication. The donkeys with an increased Do-CPS score on day three were mostly donkeys with a solar abscess (one donkey suffered from laminitis) and the parameter 'Movement' was scored in all donkeys. This corresponds with the study of Lindegaard et al. [8] were lameness score was chosen as the primary outcome measure, because lameness is most often used for evaluation of orthopedic pain in horses.

The subgroup facial pain (Fig. 4B) consists of five donkeys with eye problems (conjunctivitis, keratitis or cornea ulceration), one donkey with ulcerations in the mouth and one donkey with a fractured jaw. The increased Do-CPS scores on day two and three were from the patient with ulcerations in the mouth, this patient was treated with prednisolone. A possible explanation for the increased Do-CPS scores could be that only the prednisolone treatment did not provide enough pain relief.

In the subgroup colic pain (Fig. 4C) a donkey had a Do-CPS score of 7.5 on day one. This donkey suffered from colic of an unknown cause and was euthanized after the second pain assessment because the donkey was not comfortable despite of the analgesic treatment. Another donkey from this group, suffering from impaction colic, was euthanized after de first assessment of pain. Not every surviving donkey was observed every following day after the first assessment of pain. However, after two days all Do-CPS scores were 0. This may be because the surviving patients, suffering from different causes of colic (impaction and unknown cause), were cured after the first observation.

The mean Do-CPS scores of the subgroup post-operative pain (Fig. 4D) shows increased T = 0 pre-OK Do-CPS scores. An increase of CPS scores in prior to surgery was also seen in the study of van Loon et al.[11]. In this study, the CPS described by Bussières et al.[12] was used to assess pain in twenty horses who were admitted for castration. The study illustrated that horses did not show increased CPS scores after being subjected to standard anesthetic and analgesic protocols during surgical castration. They interpreted this as the analgesic treatment being effective in these horses. Moreover, in this study the T = 0 pre-OK CPS scores were also marginally increased. They described that this could be explained by the fact that the patient group comprised of mostly young, excitable, and inexperienced stallions which were excited by transport and new stable conditions, and thus was not related to pain. However, in the current study this patient group contained donkeys with different ages and reasons of admittance for surgery (sarcoid removal, castration and molar extraction). Although, the reason for the increased Do-CPS in prior to the surgery could also be due to excitement after transport and/or new stable conditions for these donkeys.

Because of the limited amount of data, no statistical tests for the effects over time could be conducted (Fig. 4). In a follow-up study, more data should be collected to receive more information about the relation between Do-CPS scores over time in patients. These results could be used to determine the effectiveness of the analgesic treatment. The case report of Dutton et al [13] describes the usefulness of composite pain scoring in individual horses with severe hoof pain. The study shows that pain scores facilitated the early, objective recognition of the changing pain status. In this study a modified CPS, which included a dynamic score (Obel laminitis pain scale[19]) and a static score (Equine-specific modified Glasgow composite scale[20]) was used. In a study of Regan et al.[21] the (adapted) Obel laminitis pain scale was used to assess lameness in donkeys. They concluded that the scoring system was not originally designed for use in donkeys and may not be sufficiently sensitive to detect slight changes in donkeys. However, several parameters of the Do-CPS correspond to the Obel (and Glasgow) scale and Do-CPS scoring was found to be useful for the recognition of changing pain states as well.

The study of Bussières et al. [12] showed that the key specific and most sensitive behavioral indices for acute orthopedic pain in horses were ‘Response to palpation of the painful area’, ‘Posture’ and, to a lesser extent, ‘Pawing on the floor’, ‘Kicking at the abdomen’ and ‘Head movements’. This corresponds with findings in the current study, however in the current study the parameter ‘Pawing on the floor’ also had a good sensitivity. These donkeys were pointing the limb that was painful (Fig. 5). Furthermore, the parameters ‘Weight distribution’, ‘Movement’ and ‘Reaction to palpation’ were most sensitive in the lameness subgroup. The study of van Loon and van Dierendonck [15] showed that the most sensitive parameters of the EQUUS-COMPASS for acute colic in horses were the ‘Character of borborygmi’, ‘Posture’, ‘Sweating’, ‘Reaction to observer’ and ‘Palpation of the painful flank’. In the current study the parameters ‘Overall appearance’ and ‘Digestive sounds’ were the most sensitive. For visceral post-operative pain in horses van

Fig. 5 *Jack F Eire (Pat24) suffering from lameness caused by a solar abscess showing 'pointing limb'.*

Loon et al. [14] showed that 'Pawing on the floor', 'Overall appearance', 'Head movements' and 'Interactive behavior' were the most important parameters of the CPS. In the recent study, the parameters 'Reaction to palpation' and 'Heart rate' were most sensitive in the post-operative pain group. The parameter 'Heart rate' was only scored in young stallions after surgical castration. The study of Taffarel et al.[22] evaluated the UNESP-Botucatu multi-dimensional CPS for assessing acute pain in horses after surgical castration. This study also showed an increased heart rate after surgical castration and after the study this CPS was refined. In the refined CPS the parameter 'Heart rate' will only be scored if the heart rate has increased ≥ 25% compared to the initial heart rate. However, more research has to be conducted to see if the parameter in the Do-CPS needs to be refined for this subgroup of patients. In this study no good sensitive parameters were found for the facial pain group. Further research is necessary to determine if the Do-CPS can be used for patients with facial pain.

The parameters 'Rectal temperature' and 'Pain sounds' have not been scored in any donkey. This corresponds to the review article of Ashley at al.[23] in which comparison of indicators of pain in horses and donkeys is described. According to this review article the parameters 'Rectal temperature', 'Laying down/rolling', 'Kicking at abdomen' and 'Pain sounds' have not been reported in donkeys. However, in the current study the parameters 'Laying down/rolling' and 'Kicking at abdomen' were seen in two patients, both suffering from impaction colic. It could be possible that in the future, when more data is available, the parameters 'Rectal temperature' and 'Pain sounds' can be removed from the Do-CPS.

A cut-off value of ≥ 5 was determined with the data of the study of van Overbeek (unpublished data) for differentiation between patients and healthy pain free control donkeys. In the current study, good sensitivity and specificity of Do-CPS scores (unweighted data) were found (Table 2A). Furthermore, good sensitivity and specificity (unweighted data) were found for the subgroups lameness, colic and post-operative pain. The sensitivity (unweighted data) for facial pain was low (29%). However, more research has to be conducted to conclude that the Do-CPS cannot be used for assessing acute pain in patients with facial pain.

In the current study, the data of van Overbeek (unpublished data) was used to determine sensitivity and specificity for all individual parameters of the Do-CPS and these were used to determine weighting factors (Appendix 4). By using weighting factors for individual parameters, sensitivity and specificity can be potentially improved with a limited number of parameters. The sensitivity and specificity of Do-CPS scores were determined again after using these weighting factors (Table 2B). A cut-off value of ≥ 2 was used for differentiation between patients and healthy pain free control donkeys. After applying weighting factors to the individual parameters, sensitivity of the Do-CPS improved for all different subgroups of pain, especially for the subgroup facial pain. However, specificity reduced for all different subgroups of pain, especially for the subgroup post-operative pain. More research regarding weighting factors will definitely improve the clinical applicability of the Do-CPS.

There are several limitations to this study. Firstly, observer bias cannot be totally ruled out because the observers were not blinded with regard to indications for pain assessment. Moreover, the observers were aware of the treatment plan and were involved in the clinical treatment of the patients. Secondly, some of the patients were transported for hospitalization, this could have an effect on the observations. Ideally, these donkeys should be observed (at least ones) before their transportation. Furthermore, the presence of the observers, which is inevitable in this kind of study, could have had an effect as well. In the study of Regan et al.[24] was found that 24% of the donkeys showed a negative reaction towards the observer (aggression towards or avoidance of the observer). In the current study, no aggressive behavior was seen in donkeys, nonetheless divergent behavior because of observer's presence cannot be excluded. However, this effect is comparable for all patients in this study. Finally, the number of patients in some of the subgroups was limited. For this reason, more research to investigate the usefulness and applicability of the Do-CPS in a more extended group of donkeys, especially with colic, facial and post-operative pain, is necessary.

Suggestions for improvement of the Do-CPS

After conducting the study, several suggestions to improve the Do-CPS were found and/or suggested by the veterinarians at 'the Donkey Sanctuary'.

The first suggestion for improvement involves weight shifting. Weight shifting was seen in several patients, especially in patients of the subgroup lameness. In the Do-CPS, weight shifting (mild) is only mentioned once in the parameter 'Posture' and results in a score of 1 for the Do-CPS. After observing a very painful patient (high Do-CPS score), the veterinarians at 'the Donkey Sanctuary' used the frequency of weight shifting to determine the severity of pain for this patient. This turned out to be an adequate way to assess pain in this donkey. Therefore, weight shifting could possibly benefit from being used in the Do-CPS as an individual parameter. Table 3 shows the description of the suggested scoring possibilities.

Table 3 *Improved parameter 'Weight shifting'.*

Weight shifting	**Score**
Quietly standing and/or one hind leg resting	0
Mild weight shifting (1 or 2 times/5min)	1
Frequent weight shifting (3 or 4 times/5min)	2
Excessive weight shifting (>4 times/5min)	3

The second suggestion for improvement involves the parameter 'Pawing at floor'. This parameter implies that the donkey should actually paw the floor before a score of 2 can be given. However, in the CPS as described by Bussières et al.[12] 'Pawing on the floor' is described as 'Pointing, hanging limbs'. In the Do-CPS 'Pointing limb' is a separate scoring possibility, this implies that 'pointing limb' differs from 'pawing at floor'. Table 4 shows the improved parameter 'Pawing at floor' for the Do-CPS. As mentioned, pointing the painful limb (Fig. 5) was often seen in patients from the subgroup lameness. A donkey that is pointing its limb, also has an abnormal weight distribution. This also applies to donkeys who are weight shifting. Therefore, the parameter 'Weight distribution' could be erased to increase the efficiency of the Do-CPS.

Table 4 *Improved parameter 'Pawing at floor'.*

Pawing at floor (pointing, hanging limbs)	**Score**
Quietly standing, does not paw at floor	0
Occasional pawing at floor (1 or 2 episodes /5min)	1
Frequent pawing at floor (>2 episodes/5min)	2
Extensive pawing at floor (>2 episodes/5min)	3

Lastly, the scoring possibility 'Hypermotility or steelband' needs to change into 'Hypermotility or tympanic sounds' because the veterinarians at 'The Donkey Sanctuary' were calling this sound 'tympanic'.

All the previous mentioned improvements for the Do-CPS have resulted in an improved Do-CPS (Appendix 5) that can be used to obtain more data in validation studies. However, the parameters of the Do-CPS that were changed/removed need to be scored as well, thus afterwards all the data can be combined to obtain a larger dataset.

Conclusion

In conclusion, the high interobserver reliability and significant difference between Do-CPS scores of patients and healthy pain free control donkeys were both indicative that the Do-CPS is an adequate tool to assess acute pain in donkeys, caused by lameness, facial pain, colic and post-operative pain. The Do-CPS was especially effective in patients suffering from lameness and colic pain. Furthermore, this study showed that it was not necessary to find a similar (i.e., age, gender, weight, size) control donkey for every patient. More research will undoubtedly improve the Do-CPS even more and make it suitable for use in veterinary practice.

Acknowledgements

I would like to thank Annechien Smalbroek and all the employees of 'The Donkey Sanctuary' in Sidmouth (England) for their help with data collection, 'Ezelvereniging het Nederlands Ezelstamboek (VNE)' for their financial support and of course Thijs van Loon and Machteld van Dierendonck for supervising my master thesis.

References

1. Zimmermann M. Ethical considerations in relation to pain in animal experimentation. *Acta Physiol Scand Suppl*. 1986;554:221-233.
2. Dujardin CL, van Loon JPAM. Pain recognition and treatment in the horse: a survey of equine veterinarians in The Netherlands and Belgium. *Tijdschr Diergeneeskd*. 2011;136(10):715-724.
3. Price J, Catriona S, Welsh EM, Waran NK. Preliminary evaluation of a behaviour–based system for assessment of post–operative pain in horses following arthroscopic surgery. *Vet Anaesth Analg*. 2003;30(3):124-137.
4. de Grauw JC, van Loon JPAM. Systematic pain assessment in horses. *Vet J*. 2016;209:14-22.
5. Burden F, Thiemann A. Donkeys are different. *J Equine Vet Sci*. 2015;35(5):376-382.
6. Thiemann A. Clinical approach to the dull donkey. *In Pract*. 2013;35(8):470-476.
7. Gleerup KB, Lindegaard C. Recognition and quantification of pain in horses: A tutorial review. *Equine Vet Educ*. 2016;28(1):47-57.
8. Lindegaard C, Thomsen MH, Larsen S, Andersen PH. Analgesic efficacy of intra-articular morphine in experimentally induced radiocarpal synovitis in horses. *Vet Anaesth Analg*. 2010;37(2):171-185.
9. Graubner C, Gerber V, Doherr M, Spadavecchia C. Clinical application and reliability of a post abdominal surgery pain assessment scale (PASPAS) in horses. *Vet J*. 2011;188:178-183.
10. Van Dierendonck MC, van Loon JPAM. Monitoring acute equine visceral pain with the Equine Utrecht University Scale for Composite Pain Assessment (EQUUS-COMPASS) and the Equine Utrecht University Scale for Facial Assessment of Pain (EQUUS-FAP): A validation study. *Vet J*. 2016;216(3):175-177.
11. van Loon JPAM, Back W, Hellebrekers LJ, Ren van Weeren P. Application of a Composite Pain Scale to Objectively Monitor Horses with Somatic and Visceral Pain under Hospital Conditions. *J Equine Vet Sci*. 2010;30:641-649.
12. Bussières G, Jacques C, Lainay O, et al. Development of a composite orthopaedic pain scale in horses. *Res Vet Sci*. 2008;85(2):294-306.
13. Dutton DW, Lashnits KJ, Wegner K. Managing severe hoof pain in a horse using multimodal analgesia and a modified composite pain score. *Equine Vet Educ*. 2009;21(1):37-43.
14. Van Loon JPAM, Jonckheer-Sheehy VSM, Back W, René Van Weeren P, Hellebrekers LJ. Monitoring equine visceral pain with a composite pain scale score and correlation with survival after emergency gastrointestinal surgery. *Vet J*. 2014;200:109-115.
15. Van Loon JPAM, Dierendonck MC Van. Monitoring acute equine visceral pain with the Equine Utrecht University Scale for Composite Pain Assessment (EQUUS-COMPASS) and the Equine Utrecht University Scale for Facial Assessment of Pain (EQUUS-FAP): A scale-construction study. *Vet J*. 2015;206(3):356-364.
16. Trawford AF, Crane MA. Nursing care of the donkey. *Equine Vet Educ*. 1995;7(1):36-38.
17. Robertson S. The importance of assessing pain in horses and donkeys. *Equine Vet J*. 2010;38(1):5-6.
18. Van Dijk M, De Boer JB, Koot HM, et al. The Association Between Physiological and Behavioral Pain Measures in 0-to 3-Year-Old Infants after Major Surgery. *J Pain Symptom Manage*. 2001;2222(1):600-609.
19. Obel N. Studies on the histopathology of

acute laminitis. *Stud Histopathol acute laminitis*. 1948.

20. Morton CM, Reid J, Scott EM, Holton LL, Nolan AM. Application of a scaling model to establish and validate an interval level pain scale for assessment of acute pain in dogs. *Am J Vet Res*. 2005;66(12):2154-2166.
21. Regan FH, Hockenhull J, Pritchard JC, Waterman-Pearson AE, Whay HR. Identifying behavioural differences in working donkeys in response to analgesic administration. *Equine Vet J*. 2016;48(1):33-38.
22. Taffarel MO, Luna SPL, de Oliveira FA, et al. Refinement and partial validation of the UNESP-Botucatu multidimensional composite pain scale for assessing postoperative pain in horses. *BMC Vet Res*. 2015;11(1):83.
23. Ashley FH, Waterman-Pearson AE, Whay HR. Behavioural assessment of pain in horses and donkeys: application to clinical practice and future studies. *Equine Vet J*. 2010;37(6):565-575.
24. Regan FH, Hockenhull J, Pritchard JC, Waterman-Pearson AE, Whay HR. Behavioural repertoire of working donkeys and consistency of behaviour over time, as a preliminary step towards identifying pain-related behaviours. *PLoS One*. 2014;9(7).

Appendices

Appendix 1: The Donkey Composite Pain Scale (Do-CPS) used in this study

Donkey Composite Pain Scale (Do-CPS)

Overall appearance	**Score**	**Pain sounds**	**Score**
Alert and/or is interacting with mate/group	0	No audible signs of pain	0
Mildly depressed and/or restless and/or decreased interaction with group mate/group	1	Occasional teeth grinding or moaning (1 or 2 times/5min)	1
Moderately depressed and/or aggressive or no reaction mate/group	2	Frequent teeth grinding or moaning (3 or 4 times/5min)	2
Severely depressed	3	Excessive teeth grinding or moaning (>4 times/5min)	3
Posture	**Score**	**Changes in behavior of mate/group**	**Score**
Quietly standing and/or one hind leg resting	0	Patient is in the group	0
Slightly tucked up abdomen and/or mild weight shifting	1		
Extremely tucked up abdomen and/or hunched back and/or stretching limbs/body and/or mild muscle tremors	2		
Sits on hind quarters and/or extreme muscle tremors	3	Mate/group leaves or has left patient (excluding herd behavior)	3
Weight distribution	**Score**	**Eating (present food)**	**Score**
Normal weight distribution	0	Eats normally or fasts	0
		Eats less and/or slowly	2
Abnormal weight distribution	3	Not interested in food	3
Laying down, rolling (excluding auto grooming)	**Score**	**Movement**	**Score**
Does not lie down or rests lying down	0	No reluctance to move and normal gait	0
Attempts to lie down or is lying down <50% of the time	1	Mildly abnormal gait (1 or 2 out of 5 lameness) and/or stiff walk	1
Lying down >50% of the time	2	Reluctance to move when motivated and/or severely abnormal gait (3 to 5 out of 5 lameness)	2
Lies down in abnormal position: on its side with stretched limbs or on its back and/or is rolling	3	No movement or is lying down	3
Head carriage	**Score**	**Respiratory rate**	**Score**
Ear base above withers or eats/drinks (from the ground)	0	12-28 breaths/min	0
		29-32 breaths/min	1
Ear base at the level of the withers	2	33-36 breaths/min	2
Ear base below the withers	3	>36 breaths/min	3
Position of the ears (>75% of the time)	**Score**	**Reaction to observer(s)**	**Score**
Normal position	0	Reaction to observer(s)	0
		Mild reaction to observer(s)	2
Abnormal position (backwards/sideways/flat)	3	No reaction to observer(s)	3
Episodes of tail flicking (excluding flicking to insects)	**Score**	**Reaction to palpation of the painful area**	**Score**
No tail flicking, tail in normal position	0	No reaction to palpation	0
Occasional tail flicking (1 or 2 episodes/5min)	1		
Frequent tail flicking (3 or 4 episodes/5min)	2	Mild reaction to palpation	2
Excessive tail flicking (>4 episodes/5min) and/or lifts out tail or tails is tucked in	3	Severe reaction to palpation	3
Kicking at abdomen	**Score**	**Heart rate**	**Score**
Quietly standing, no kicking	0	32-52 beats/min	0
Looking at abdomen	1	53-60 beats/min	1
Lifting up hind legs, may kick once or twice at abdomen	2	61-68 beats/min	2
Extensive kicking at abdomen (>2 episodes/5min)	3	>68 beats/min	3
Pawing at floor	**Score**	**Rectal temperature**	**Score**
Quietly standing, does not paw at floor	0	35.7°C – 38.0°C	0
Points limb	1	35.3°C – 35.6°C or 38.1°C – 38.5°C	1
Occasional pawing at floor (1 or 2 episodes/5min)	2	34.7°C – 35.2°C or 38.6°C – 39.0°C	2
Extensive pawing at floor (>2 episodes/5min)	3	<34.6°C or >39.1°C	3
Sweating	**Score**	**Digestive sounds**	**Score**
No signs of sweating	0	Normal motility	0
		Decreased motility	1
Signs of sweating (wet spots visible, no droplets or streams)	2	No motility	2
Excessive sweating (streams or droplets)	3	Hypermotility or steelband	3
Total composite pain score			**60**

Appendix 2: The CPS for donkeys used in the study of van Overbeek (unpublished data)

Composite Pain Scale for donkeys

Physiological data

Heart rate	**score**
32-52 beats/min	0
53-60 beats/min	1
61-68 beats/min	2
>68 beats/min	3
Respiratory rate	**score**
12-28 breaths/min	0
29-32 breaths/min	1
33-36 breaths/min	2
>36 breaths/min	3

Temperature	**score**
35.7 – 38.0	0
35.3 – 35.6 or 38.1 – 38.5	1
34.7 – 35.2 or 38.6 – 39.0	2
34.2 – 34.6 or 39.1 – 39.5	3
Digestive sounds	**score**
Normal motility	0
Decreased motility	1
No motility	2
Hypermotility or steelband	3

Behavioural data

Overall appearance	**score**
Alert and/or is interacting with mate/group	0
Mildly depressed and/or restless and/or decreased interaction with mate/group	1
Moderately depressed and/or aggressive or no reaction to mate/group	2
Severely depressed	3
Posture	**score**
Quietly standing and/or one hind leg resting	0
Slightly tucked up abdomen and/or mild weight shifting	1
Extremely tucked up abdomen and/or hunched back and/or stretching limbs/body and/or mild muscle tremors	2
Sits on hind quarters and/or extreme muscle tremors	3

Weight distribution	**score**
Normal weight distribution	0
Abnormal weight distribution	3
Laying down, rolling	**score**
Does not lie down or rests lying down	0
Attempts to lie down or is lying down less than 50% of the time	1
Lying down 50% of the time or more	2
Lies down in abnormal position: on its side with stretched limbs or on its back and/or is rolling	3

Head carriage	**score**
Head in normal position	0
Lower carriage of the head	1
Head carriage below the withers	2
Head carriage below the withers + swelling of the muzzle	3
Position of the ears	**Score**
Forwards/straight	0
Sideways/flat	1
	2
Backwards/flat	3
Episodes of tail flicking (excluding flicking to chase off insects)	**score**
No tail flicking, tail in normal position	0
Occasional tail flicking (one or two episodes/5min)	1
Frequent tail flicking (three to four episodes/5min)	2
Excessive tail flicking (more than five episodes/5 min) and/or lifts out tail or tail is tucked in	3
Kicking at abdomen	**score**
Quietly standing, no kicking	0
Looking at abdomen	1
Lifting up hind legs, may kick once or twice at abdomen	2
Extensive kicking at abdomen	3
Pawing at floor (number of episodes)	**score**
Quietly standing, does not paw at floor	0
Points limb	1
Occasional pawing at floor (one or two episodes/5 min)	2
Extensive pawing at floor	3
Sweating	**score**
No signs of sweating	0
Warm or damp to touch, no sweat or wet spots visible	1
Wet spots visible, no droplets of streams	2
Excessive sweating, may include streams or droplets	3

Pain sounds	**score**
No audible signs of pain	0
Occasional teeth grinding or moaning (once or twice/5 min)	1
Frequent teeth grinding or moaning (three to four times/5 min)	2
Excessive teeth grinding or moaning (more than five times/5 min)	3
Changes in behavior of mate/group	**score**
Patient is in the group	0
Mate/group leaves or has left patient (excluding herd behavior)	3
Eating	**score**
Eats normally or fasts	0
Hesitates to eat	1
Eats less or pretends to eat	2
Not interested in food	3
Reaction to observer(s)	**score**
Reaction to observer and/or ear movement towards observer	0
Mild reaction to observer and/or less ear movement	2
No reaction to observer(s) and/or no ear movement	3
Movement	**score**
No reluctance to move and normal gait	0
No reluctance to move and/or mildly abnormal gait	1
Reluctance to move when motivating with treat and/or severely abnormal gait	2
No movement or is lying down	3
Reaction to palpation of painful area	**score**
No reaction to palpation	0
Mild reaction to palpation	1
Moderate (resistance) reaction to palpation	2
Severe (aggressive) reaction to palpation	3

Appendix 3: Patient list

Patient	**Donkey code**	**Name**	**Gender**	**Age**	**Medical condition**	**Patient group**
1	Pat01	Sparkle Walker	Jenny / mare	27	Solar abcess	Lameness
2	Pat02	Dolly KS	Jenny / mare	23	Laminitis	Lameness
3	Pat05	Jack Tidball	Gelding	21	Mouth ulcerations	Facial pain
4	Pat06	Zebedee Montague	Gelding	23	Solar abcess	Lameness
5	Pat07	Curry Walsh	Jack / stallion	1	Castration	Post-operative pain
6	Pat08	Ralph Walsh	Jack / stallion	1	Castration	Post-operative pain
7	Pat09	Beauty Walks	Jenny / mare	14	Solar abcess	Lameness
8	Pat10	William Woodland	Gelding	22	Solar abcess	Lameness
9	Pat11	Rosschap Eire	Gelding	8	Ostheoarthritis	Lameness
10	Pat12	Archie Keevans	Gelding	17	Keratitis	Facial pain
11	Pat13	Peter C	Gelding	28	Corneal ulceration	Facial pain
12	Pat14	Patsey Eire	Gelding	25	Solar abcess	Lameness
13	Pat15	Coco Culling	Gelding	31	Solar abcess	Lameness
14	Pat16	Crackers Hall	Gelding	37	Laminitis and white line abcess	Lameness
15	Pat17	Rosa Lewis	Jenny / mare	18	Ostheoarthritis	Lameness
16	Pat19	Edward Hancock	Gelding	6	Solar abcess	Lameness
17	Pat20	Tayto	Gelding	14	Conjunctivitis and uveitis	Facial pain
18	Pat21	Mr McGregor Eire	Gelding	28	Impaction colic	Colic
19	Pat22	Penny Starsmore	Jenny / mare	17	Solar abcess	Lameness
20	Pat23	Paddy Stevens	Gelding	9	Impaction colic	Colic
21	Pat24	Smokey Stiles	Gelding	18	Impaction colic	Colic
22	Pat25	Jack F Eire	Gelding	23	Solar abcess	Lameness
23	Pat26	Malty Eire	Gelding	26	Laminitis	Lameness
24	Pat27	Ciara Eire	Jenny / mare	9	Solar abcess	Lameness
25	Pat28	Cocoa Clews	Gelding	22	Impaction colic	Colic
26	Pat30	Ganty Eire	Gelding	7	Laminitis	Lameness
27	Pat31	Fourmay Ake	Jenny / mare	16	Solar abcess	Lameness
28	Pat32	Sile M Eire	Jenny / mare	8	Conjunctivitis	Facial pain
29	Pat33	Willie Drennan Eire	Gelding	9	Sarcoid removal	Post-operative pain
30	Pat34	Oreo	Jack / stallion	1	Castration	Post-operative pain
31	Pat35	Buzz MI	Jenny / mare	29	Other colic	Colic
32	Pat36	Sandon Proud Playboy	Gelding	23	Molar removal	Post-operative pain
33	Pat37	Violet Davies	Jenny / mare	25	Painful frog	Lameness
34	Pat38	Charlie Chuck Devlin	Gelding	9	Solar abcess	Lameness
35	Pat39	Abbie Eire	Jenny / mare	26	Other colic	Colic
36	Pat40	Brodaha Eire	Jenny / mare	8	Solar abcess	Lameness
37	Pat41	Jacko Buttle	Gelding	17	Hoofwall bruise	Lameness
38	Pat42	Cobweb PSNI	Jenny / mare	5	Solar abcess	Lameness
39	Pat43	Henry Gribben	Gelding	13	Corneal ulceration	Facial pain
40	Pat44	Eey-ore Eire	Gelding	13	Unknown cause	Lameness
41	Pat45	Rodney Barrett	Gelding	10	Other colic	Colic
42	Pat46	Camalan Jester	Gelding	15	Fractured jaw	Facial pain
43	Pat47	Bruno D Eire	Gelding	9	Sarcoid removal	Post-operative pain
44	Pat48	Phoebe Wing	Jenny / mare	26	Solar abcess	Lameness

Appendix 4: Weighting factors of individual parameters of the Do-CPS

Data of T. van Overbeek (unpublished data)	**All data (n = 68)**	**Lameness (n = 35)**	**Facial pain (n = 10)**	**Colic (n = 10)**	**Post-op (n = 13)**
Overall appearance	2	2	1	3	2
Posture	1	1	0	3	0
Weight distribution	2	3	0	1	0
Laying down, rolling	0	0	0	0	0
Head carriage	0	0	0	1	0
Position of the ears	1	1	1	1	0
Tail flicking	1	0	2	1	1
Kicking at abdomen	0	0	0	0	0
Pawing at floor	0	0	0	1	0
Sweating	0	0	0	1	0
Pain sounds	0	0	0	0	0
Changes in behaviour	0	0	0	1	0
Eating	0	0	0	2	0
Movement	2	3	0	1	0
Respiratory rate	1	0	0	1	2
Reaction to observer(s)	1	0	1	2	1
Reaction to palpation	3	3	2	3	2
Heart rate	0	1	0	0	1
Rectal temperature	0	0	0	0	0
Digestive sounds	1	0	1	3	0

Post-op = Post-operative pain.

When the percentage of sensitivity or specificity of the parameter was ≤ 25%, a weighting factor of 0; between 25% and 50%, a weighting factor of 1; between 50% and 25%, a weighting factor of 2; and when ≥ 75% a weighting factor of 3 is applied.

Appendix 5: Improved Donkey Composite Pain Scale (Do-CPS) after this study

Donkey Composite Pain Scale (Do-CPS)

Overall appearance	**Score**	**Pain sounds**	**Score**
Alert and/or is interacting with mate/group	0	No audible signs of pain	0
Mildly depressed and/or restless and/or decreased interaction with group mate/group	1	Occasional teeth grinding or moaning (1 or 2 times/5min)	1
Moderately depressed and/or aggressive or no reaction mate/group	2	Frequent teeth grinding or moaning (3 or 4 times/5min)	2
Severely depressed	3	Excessive teeth grinding or moaning (>4 times/5min)	3
Posture	**Score**	**Changes in behavior of mate/group**	**Score**
Quietly standing and/or one hind leg resting	0	Patient is in the group	0
Slightly tucked up abdomen	1		
Extremely tucked up abdomen and/or hunched back and/or stretching limbs/body and/or mild muscle tremors	2		
Sits on hind quarters and/or extreme muscle tremors	3	Mate/group leaves or has left patient (excluding herd behavior)	3
Weight shifting	**Score**	**Eating (present food)**	**Score**
Quietly standing and/or one hind leg resting	0	Eats normally or fasts	0
Mild weight shifting (1 or 2 times/5min)	1		
Frequent weight shifting (3 or 4 times/5min)	2	Eats less and/or slowly	2
Excessive weight shifting (>4 times/5min)	3	Not interested in food	3
Laying down, rolling (excluding auto grooming)	**Score**	**Movement**	**Score**
Does not lie down or rests lying down	0	No reluctance to move and normal gait	0
Attempts to lie down or is lying down <50% of the time	1	Mildly abnormal gait (1 or 2 out of 5 lameness) and/or stiff walk	1
Lying down >50% of the time	2	Reluctance to move when motivated and/or severely abnormal gait (3 to 5 out of 5 lameness)	2
Lies down in abnormal position: on its side with stretched limbs or on its back and/or is rolling	3	No movement or is lying down	3
Head carriage	**Score**	**Respiratory rate**	**Score**
Ear base above withers or eats/drinks (from the ground)	0	12-28 breaths/min	0
		29-32 breaths/min	1
Ear base at the level of the withers	2	33-36 breaths/min	2
Ear base below the withers	3	>36 breaths/min	3
Position of the ears (>75% of the time)	**Score**	**Reaction to observer(s)**	**Score**
Normal position	0	Reaction to observer(s)	0
		Mild reaction to observer(s)	2
Abnormal position (backwards/sideways/flat)	3	No reaction to observer(s)	3
Episodes of tail flicking (excluding flicking to insects)	**Score**	**Reaction to palpation of the painful area**	**Score**
No tail flicking, tail in normal position	0	No reaction to palpation	0
Occasional tail flicking (1 or 2 episodes/5min)	1		
Frequent tail flicking (3 or 4 episodes/5min)	2	Mild reaction to palpation	2
Excessive tail flicking (>4 episodes/5min) and/or lifts out tail or tails is tucked in	3	Severe reaction to palpation	3
Kicking at abdomen	**Score**	**Heart rate**	**Score**
Quietly standing, no kicking	0	32-52 beats/min	0
Looking at abdomen	1	53-60 beats/min	1
Lifting up hind legs, may kick once or twice at abdomen	2	61-68 beats/min	2
Extensive kicking at abdomen (>2 episodes/5min)	3	>68 beats/min	3
Pawing at floor (pointing, hanging limbs)	**Score**	**Rectal temperature**	**Score**
Quietly standing, does not paw at floor	0	35.7°C – 38.0°C	0
Occasional pawing at floor (1 or 2 episodes/5min)	1	35.3°C – 35.6°C or 38.1°C – 38.5°C	1
Frequent pawing at floor (>2 episodes/5min)	2	34.7°C – 35.2°C or 38.6°C – 39.0°C	2
Extensive pawing at floor (>2 episodes/5min)	3	<34.6°C or >39.1°C	3
Sweating	**Score**	**Digestive sounds**	**Score**
No signs of sweating	0	Normal motility	0
		Decreased motility	1
Signs of sweating (wet spots visible, no droplets or streams)	2	No motility	2
Excessive sweating (streams or droplets)	3	Hypermotility or tympanic sounds	3
Total composite pain score			**60**