Characterization of a reversible lameness model in the horse

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Introduction

Horses may be subject to prolonged periods of lameness associated with naturally occurring conditions including trauma, osteoarthritis and laminitis. In the aforementioned conditions, lameness is attributed to pain which, if the pain becomes persistent, modifies the nervous system physiology and anatomy leading to upregulated pain levels and self-perpetuating sensory stimulation. Persistent pain can lead to an overall decrease in the quality of life with reduced appetite and prolonged periods of recumbency. Changes within the nervous system accompanying persistent pain decrease the effectiveness of analgesic protocols normally useful for acute nociceptive pain. Alterations in the anatomy and physiology of neurons associated with persistent pain have been documented in the horse. Horses suffering from refractory laminitis have a significant decrease in the number of myelinated and unmyelinated nerve fibres in the lateral palmar nerve as compared to controls. These findings are consistent with a chronic pain state with a neuropathic component. Extrapolation of data from acute pain models for use in clinical persistent pain situations is problematic because the mechanisms are much more complex in persistent pain cases, and pain control regimens developed using acute pain models do not directly address neuropathic or centrally mediated pain.

Current equine pain models typically involve the application of a noxious stimulus (mechanical, chemical, thermal, electrical) and some measure of reaction for a short period of time. A model which creates prolonged pain for the duration of 120 hours would enable evaluation of analgesic agents which require multiple dosages to reach effective circulating plasma levels, optimum dosing intervals for analgesic agents with short circulating half-life, and would provide a gateway for further studies utilizing the developed model for a longer duration model of persistent pain.

An acute equine lameness model has been developed by applying sole pressure to produce a mechanical lameness with use of a modified shoe and adjustable set screw. These studies have all been limited to short periods of time (2 – 9 hours) and can result in sole abscission with weekly application in as little as three to four consecutive weeks. We proposed to utilize a tightened T-clamp around the distal circumference of the hoof wall to provide constant pressure to the hoof capsule for...
five days. We hypothesized that the model would produce a lameness that is repeatable in severity between and within a horse, and also prolonged, quantifiable, and reversible as determined by subjective and kinetic gait analysis for five consecutive days. The overall objective was to improve the welfare of horses with persistent lameness. With this information, we can then explore analgesic regimens aimed at the treatment of clinical cases with prolonged lameness.

Materials and methods
Animals and experimental design
Eight horses including four American Paint Horses, two Thoroughbreds, one Quarter Horse and one Haflinger from two to 12 years of age were used for the study. The median weight was 505.5 kg (range 465–580 kg). To be included, each horse was determined to be healthy based on results of physical examination, including the assessment of heart rate, respiratory rate, temperament, rectal temperature, and digital pulses. They were also determined to be free of lameness as determined by subjective and kinetic gait assessment. Physical inspection of each front foot was performed to exclude horses with divergent growth rings, prolapsed soles, and defects at the coronary band.

The front feet were trimmed and shod. The heel portion of the shoe extended 1 cm palmar from the weight bearing surface of the foot to prevent the T-clamp from contacting the ground. The caudal aspect of the clamp was reduced in width to prevent contact with the coronary band at the heel. Stainless steel pipe clamps measuring 450, 500 or 550 mm in diameter were fitted around the circumference of the foot. Epoxy was applied to the hoof wall and over the top of the clamp in order to secure the T-clamp in place and prevent proximal displacement of the clamp. The front third of the hoof wall compression. Gait assessment was repeated immediately after clamp removal (120 hrs) and then again at 144 and 168 hours (0, 24, and 48 hours following clamp release). The baseline assessments and trial were then repeated immediately for the contralateral limb after the horses had returned to baseline soundness. A 120 hour period of induced lameness was selected based on a balance between humane care for the subjects and the need for a persistent period of lameness to evaluate potential therapeutics. It was acceptable from both standpoints in the first two horses studied and was thus continued throughout the study. This period of time will allow for the evaluation of a variety of drug classes currently and potentially used in horses.

Animal care
Horses were housed in individual stalls with wood shaving bedding over rubber mats, fed grass-alfalfa mix hay twice daily, and allowed free access to water. Daily monitoring of feed and water intake, general attitude, urination, and defaecation was performed. All experimental procedures were approved by the Iowa State University Institutional Animal Care and Use Committee. Visual assessment was the primary determinant of the initial adjustment because it was predetermined this was the best way to account for the welfare of the horse. The same person (CW) performed all of the subjective lameness evaluations to ensure the welfare of the horses throughout the trial. The primary outcome was the objective force platform gait analysis, therefore blinding was not deemed necessary to validate the model. The clamp was adjusted by one person (DT) based on the subjective grade given. Subjective lameness scores were graded using a pre-
were evaluated and modified as needed in three horses. The next eight horses were subjected to the entire procedure and data collection protocol. The remaining horse was not enrolled.

All data were entered into a spreadsheet and imported into a statistical software package for analysis. The responses of treatment versus control for each outcome, ASI, \( \frac{\text{PVF}_{\text{control}} - \text{PVF}_{\text{treated}}}{\text{Impulse}_{\text{control}} - \text{Impulse}_{\text{treated}}} \), lameness score, and heart rate were analysed using linear mixed models (GLIMMIX). The objective of the analysis was to study the effect of the clamp over time.

The linear mixed-effect model, a repeated-measures analysis of variance specifically, included time, limb (left or right), order of treatment (first or second limb on the horse to have the clamp tightened), and time-order interactions as fixed effects, whereas horse was used as a random effect.

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### Gait analysis

Force platform gait analysis was performed with a 0.9 m x 0.6 m biomechanical stationary platform embedded in the centre of a 40 m walkway. Velocity and acceleration were obtained by five photoelectric cells spaced 0.5 m apart at the level of the horse’s shoulder. A computer analysis system recorded data from each foot strike. A valid footfall consisted of the entire front foot striking the plate followed by the entire ipsilateral hind foot striking the plate; each foot strike must be accompanied by appropriate ground reaction force-time curves. Valid footfalls also required the subject to have a calculated velocity between 2.5 and 3.5 m/sec and an acceleration less than \( \pm 0.5 \text{ m/sec}^2 \). Horses were trotted in hand to obtain five valid footfalls for both forelimbs at each sampling period. The PVF (N/kg) and impulse (total force applied over time; Ns/kg) were recorded for each foot strike and normalized for weight as Newtons per kilogram body weight. The ASI was calculated as the absolute value of:

\[
\text{ASI} = \frac{\text{PVF}_{\text{left}} - \text{PVF}_{\text{right}}}{0.5 \left( \frac{\text{PVF}_{\text{left}} + \text{PVF}_{\text{right}}}{2} \right)} \times 100\% \quad (13).
\]

### Statistical analysis

It was not possible to calculate a sample size because there were not any data available for this model. Twelve horses were approved by the Iowa State University Animal Care and Use Committee for use. The technical aspects of the model, shoe and clamp placement, acrylic, and clamp tightening were evaluated and modified as needed in three horses. The next eight horses were subjected to the entire procedure and data collection protocol. The remaining horse was not enrolled.

All data were entered into a spreadsheet and imported into a statistical software package for analysis. The responses of treatment versus control for each outcome, ASI, \( \frac{\text{PVF}_{\text{control}} - \text{PVF}_{\text{treated}}}{\text{Impulse}_{\text{control}} - \text{Impulse}_{\text{treated}}} \), lameness score, and heart rate were analysed using linear mixed models (GLIMMIX). The objective of the analysis was to study the effect of the clamp over time.

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**Figure 2**

Mean subjective lameness grade (± SE) over time for the limb treated first (■) compared to the limb treated second (●). There was a significant interaction (p <0.0001) of the subjective lameness grade and time. Subjective lameness grade was significantly related to time. The data points between the solid vertical lines are when the clamp was tight around the hoof wall.

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points for each limb. The linear mixed model is appropriate to use when there are both fixed and random effects, and will work with small sample sizes for evaluating fixed effects (14).

The least-square means and associated standard errors estimated from the model were calculated for each treatment. Differences in time points were assessed using two-tailed pairwise Tukey’s t-tests. Values of $p \leq 0.05$ were considered significant.

Results

Subjective evaluation and heart rate

Peak mean lameness grade ± standard error was $2.75 \pm 0.15$, as seen on the second treated limb at 24 hours following clamp tightening. The mean lameness grade following clamp removal (121 hours) was $1.38 \pm 0.15$. There was a significant effect of time on lameness score ($p < 0.0001$), but no difference in limbs treated first or second was seen, as shown in Figure 2. All of the subjects were graded subjectively as being sound within 96 hours of clamp removal. Seven out of sixteen trials found the horse pointing the treated limb at the 12 hour time point; nine at 24 hours, seven at 48 hours, and four at 72 hours. No other behavioural changes were noted. Heart rate was significantly affected by time ($p = 0.0041$) and treatment order ($p = 0.0081$) (Figure 3). The heart rates increased when the clamp was tightened, however the elevation was not sustained as high or as long with the second limb to be evaluated.

Gait analysis

There were significant effects of time ($p < 0.0001$) and order of treatment ($p = 0.0112$) for ASI (Figure 4). There was a significant effect of time for all time points after the clamp was tightened for both the first and second limbs treated through and including one hour after clamp removal (121 hours). These data are shown in Table 1. These data indicate a significant effect on ASI of both having the clamp tightened (time) and the order the limb was treated, with the second limb having a higher ASI than the first. A relatively rapid return to soundness occurred with the mean ASI below five percent for both groups at 168 hours. The results were similar for the differences of PVF and impulse between treated and control limbs for time ($p < 0.0001, p < 0.0001$) and order of treatment ($p = 0.0020, p = 0.006$), respectively. There were no significant effects of limb (left or right) on time-order interactions for the outcome variables. All of the subjects were objectively judged as being sound (ASI <5.0 and PVF >9.1 N/Kg) within 96 hours following clamp removal. One of the horses used was euthanatized following the study due to reasons unrelated to the study; one horse was sold and lost to follow-up; the remaining six subjects did not show any signs of adverse effects for a minimum of one year following the study.
Discussion

To improve the welfare of horses with persistent lameness, a model to create a persistent lameness measurable by objective lameness assessment that is reversible within a short period of time is needed. This T-clamp model met our objectives. The five day period in which the T-clamp was in place was selected to demonstrate feasibility of the model with sensitivity for the subject’s welfare considerations. The degree of lameness increased immediately after clamp tightening and peaked at 24 hours. There was a gradual decrease in lameness level from 24 hours until the clamp was released at 120 hours. The day after the clamp was released, there was no significant difference from the initial baseline measurements.

The decrease in lameness from 24 to 120 hours was not expected. It was considered that the pain may increase over time because of a ramping up of response from unmyelinated nerve fibres with the application of a consistent noxious stimulus. Possible explanations may be that the construct lost compression over time, or the subjects became habituated to the hoof compression. The change over time was consistent, and in properly controlled studies, this gradual decline in lameness amplitude can be taken into account.

The significant difference between the first and second limb being treated, with the second limb being more lame than the first, was not expected. We suspect this is due to investigator actions. Our objective was to tighten the clamp to create a grade 2.5/5 lameness. When the horses returned to soundness after the first clamp was loosened on the first limb, and the investigators knew that level of lameness was safely tolerated, we aimed to achieve an equal or greater degree of subjective lameness as seen in the first limb treated, resulting in the increase in objective and subjective lameness parameters in the limb treated second. It is also possible that there is a behavioural response or hypersensitivity in that when pain was removed from one limb, then a few days later pain was applied to the opposite limb, that the horse had a more dramatic response in the second limb. Future investigations will need to be aware of this and determine if it was investigator related or a true physiological response. Furthermore, future studies could be improved by utilizing an initial adjustment period. In one session the clamp could be tightened and the horse trotted to evaluate the lameness and then either tightened or loosened to a specific ASI in the 20–30% range.

There were additional considerations with the device that were considered to improve the model. These heavy duty clamps have a fine thread mechanism to tighten, and they tightened to the desired degree of lameness with little perceptible change in torque. Because there was no subjective increase in torque when the desired lameness was achieved, and also a previous study indicated that torque applied to the clamp is an unreliable method to measure clamp load, this was not quantified or pursued (15). Clamp placement was horizontal rather than being angulated dorsally around the centre of the hoof capsule. When angulated dorsally, the construct tended to simply create heel pressure and compress the heel bulbs potentially leading to heel trauma over a 120 hour period.

The increase in lameness within the first 24 hours (Figure 2, Figure 4) was hypothesized to be due to peripheral sensitization initiated by inflammation of the lamina and secondary activation of unmyelinated nerve fibres at the site of the clamp placement. A decrease in the lameness severity was seen immediately following T-clamp removal, suggesting the lameness is the result of clamp compression,
and not trauma from the initial clamp placement. The short duration (<48 hours in some cases) required for the subjects to return to subjective and objective soundness confirms that the model is reversible. It is unlikely that any ischaemic damage to the lamina occurred due to the rapid return to soundness in all of the treated limbs, and there was an absence of long-term side effects within the study horses. The five day period was chosen as a viable starting point that was an acceptable time period for animal welfare concerns.

Though not dramatic, an increase in resting heart rate was significantly associated with both time and order of treatment. The increase associated with time is in direct relation to the lameness grade associated with the model. In this study, the first limb treated was associated with a higher resting heart rate compared to the second, and the second had increased lameness compared to the first limb treated. This is opposite what would be expected if heart rate were solely related to pain. There was probably a learned behaviour that as the study progressed, the horses were adapted to the investigators and having the heart rate measured; any increase from nervousness abated during the trial which was evident by the decrease associated with the second limb. The use of a cardiotachometer to determine heart rate without contact with the horses would probably provide a more accurate measurement. Other physiological parameters were not pursued due to the inability to accurately quantify levels of pain. Cortisol concentrations have been measured in other pain studies, but have been found to poorly quantify the level of tissue insult (16).

In developing this model, there was a requirement for the use of sound horses. A previous study found a cut-off value of 9.1 N/Kg in forelimb PVF to have a sensitivity and specificity of 0.813 in determining sound versus lame horses (12). Selective pressure application to the heel of the foot may have exacerbated previously existing soft tissue or navicular bone changes within the palmar aspect of the foot. We would recommend that the criteria of a PVF of greater than 9.1 N/kg be part of the inclusion criteria.

In this study, the subjective lameness evaluation was not blinded. The primary outcome was the objective evaluation with the force platform. The significant correlation between the subjective evaluation and kinetic gait analysis was similar to results of other studies utilizing kinetic and subjective lameness evaluation (11, 12). Subjective evaluation was performed immediately prior to the kinetic gait analysis, and was not influenced by the objective lameness evaluation.

This technique would appear to be a reproducible model of reversible persistent lameness in the horse. There are ways to potentially improve the model, including refinement of adjustments to the clamp, and measures to maintain a more persistent lameness over the study period. While a study period of 120 hours will provide more information than acute studies, it is not likely that there are notable neuropathic changes within that timeframe.

**Acknowledgements**

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**Conflict of interest**

There are no conflicts of interest to declare by the authors, nor was any external funding provided for the project.

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**Table 1**

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