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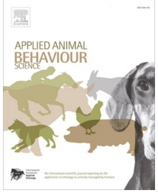

Exploring stable-based behaviour and behaviour switching for the detection of bilateral pain in equines

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ABSTRACT

Efficient and sensitive animal pain detection approaches are increasingly studied with the goal of improving animal welfare and monitoring the efficacy of treatment and rehabilitation. The aim of this study was to determine the potential of various behaviours as sensitive indicators of subtle inflammation states in equines. The long-term goal of this research is to understand how to objectively and remotely classify behaviours that are associated with inflammation using wearable inertial sensor technologies. This study represents a proof-of-concept investigation to ascertain what behavioural indices might be important in long-term monitoring of mild bilateral inflammation and recovery with a view to translating the approach to a technology-enabled remote monitoring paradigm. Bilateral synovitis of the intercarpal joints was induced in seven equines using lipopolysaccharide (0.25 ng) at time zero. The horses were confined to stables and monitored intermittently over seven days by stable-fixed video cameras. White blood cell count, carpal circumference and food availability were recorded across the study. An ethogram was created to manually annotate behaviours from video footage following lameness induction at seven different timepoints across a 1-week period. Behaviour data were processed extracting the duration, frequency and variability of behaviours. One-way repeated measures ANOVA revealed a significant time effect for white blood cell count and behaviour switching. There were no significant changes in carpal circumferences and heart rate measures over the sampling period. Food availability appears to be an important contextual factor that should be considered in pain-related behavioural studies. We conclude that behaviour variability may be a promising indicator of subtle bilateral inflammation which should be further explored in larger controlled trials and different pain presentations. Future work will seek to optimise grouping of behaviours associated with inflammation that can be detected using wearable technologies for future remote monitoring protocols.

1. Introduction

Evaluation of pain in equines is notoriously difficult yet a fundamental aspect of clinical decision making. Recently the focus of equine pain detection has expanded towards grading of equine facial expressions (Gleerup et al., 2015) supported by clinical indicators. Pain scales have been developed to include the Horse Grimace Scale (HGS), EQUUS−COMPASS and EQUUS-FAP pain scales (Dalla Costa et al., 2016) (VanDierendonck and van Loon, 2016). The HGS splits the horse's face into six sections, known as 'Facial Action Units' (Dai et al., 2020). The units are then scored independently. Scores are based on facial changes including ear position, above eye and jaw tension, and changes in mouth/chin behaviour and movement (Coneglian et al., 2020). The HGS has been useful in detecting pain related to dental disorders and laminitis. However, the HGS has not been validated for widespread use, may indicate fear (Dalla Costa et al., 2017) and likely requires comprehensive training to improve scoring accuracy (Dai et al., 2020). Further to this, a clear view of the horse's face must be available, where the Facial Action Units cannot be obstructed by typical handling tools such as a headcollar.

The composite pain scale (CPS) was developed for orthopaedic pain under an induced lameness model (Bussières et al., 2008), created in an effort to reduce rater perception and improve reliability. Gleerup and Lindegaard (2016) stated that the CPS takes approximately 10 minutes

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to administer - too long for a busy equine practice. They developed a universal pain scale, the common equine pain scale which could be administered in two minutes. The CPS was used to develop the EQUUS−COMPASS scale, intended to improve the assessment of pain associated with acute colic (van Loon and Van Dierendonck, 2015). The EQUUS-FAP scale is another pain face recognition scale incorporating nine, instead of six, facial compartments. Both the EQUUS−COMPASS and EQUUS-FAP scales have been validated for use in horses with acute colic and have been deemed suitable for use by owners as they remain sensitive to acute pain on removal of physiological parameters (Van-Dierendonck and van Loon, 2016). These scales have improved our understanding of pain, however the assessment requires time and experience, given the subjective judgements involved.

Physiological parameters in horses have weak to moderate capability to detect pain related changes while behaviour is deemed a much stronger indicator of pain (Gleerup and Lindegaard, 2016). Changes in gross physical behaviours have been investigated as indicators of health status in human and animal research (Atallah and Yang, 2009; Alsaaod et al., 2015; de Grauw and van Loon, 2016). Equine activity budgets involve the measurement of behaviour frequency and duration using intermittent video annotation, continuous video annotation or accelerometery. These measures appear sensitive to mild pain and effects of treatment (Price et al., 2003; Pritchett et al., 2003; de Grauw and van Loon, 2016; Everett et al., 2018). However, video-based methods are time consuming and have precluded real-time pain detection, discouraging their application in clinical or performance settings.

The long-term goal of our research is to understand how best to monitor subtle levels of inflammation in equines using wearable sensor technology. A wireless monitoring system could remove the human resource required by existing composite scales and enable decisions that are based on many more hours of behavioural data than is common in current practice. Such a system could be used for early detection of injury/illness risk and monitor recovery. Exploiting technology for data-driven decision making has been recognised as a frontier within the field of equine movement analysis (Bosch et al., 2018; Bragança et al., 2018; Egan et al., 2019). In order to understand what movement features might be worth targeting for longitudinal monitoring of inflammation using wearable inertial sensors, we undertook a proof-of-concept study that monitored the stable behaviours of seven equines following the induction of mild bilateral lameness. This study is based on video annotation however, our focus was specifically on behaviours that could be detectable by wearable inertial sensors with future remote-monitoring applications in mind. Thus, suitable behaviours that can provide important information about levels of inflammation needed to be identified in the first instance. In recent use, “proof-of-concept'' describes research in the beginning stages, at the cutting edge of new applications or technologies. It describes evidence, usually derived from a pilot project, that demonstrates that a design concept is feasible or as research that establishes a prototype (Kendig, 2016). The purpose of this study was to identify possible behavioural features that change with mild inflammation that could feasibly be incorporated into a longitudinal monitoring system based on wearable technology.

Bilateral lameness was induced using the established transient lipopolysaccharide (LPS) model and recovery was tracked over one week (De Grauw et al., 2009a; de Grauw et al., 2009b; Van Loon et al., 2010; de Grauw et al., 2014; Sladek et al., 2018). Currently, few published studies incorporate behavioural aetiology alongside bilateral LPS models. Van Loon et al. (2010) documented that horses spent more time in recumbency, reduced limb loading and spent less time eating in the 4–8 hour timepoints following unilateral LPS administration. It was thus expected that the equines would experience a typical ‘inflammation peak’ previously associated with induced lameness models. We discuss the merits and challenges of identifying inflammation-related behaviours at mild levels of inflammation and how they may be harnessed for future longitudinal monitoring applications. Bilateral lameness increases case complexity as typical kinematic asymmetry parameters cannot be used as a diagnostic indicator due to the apparent asymmetry between left and right sides, a parameter frequently used to detect and discern the severity of unilateral lameness. It is important to explore other non-invasive indicators of this condition. Thus, this study documents changes in gross behaviours alongside subtle levels of bilateral lameness and subsequent recovery – a situation that is difficult to discern using once-off subjective assessments in the applied field. We sought to identify behaviours that exhibited the greatest within-horse change over time as potential targets for an objective long-term monitoring system.

2. Methods

2.1. Participants

This study was approved by the University College Dublin Animal Research Ethics Committee (AREC-16−29-Brama) and the Health Products Regulation Authority (AE18982/P105) in compliance with Irish legislation on animal experiments. This experiment was part of a larger study investigating recovery from induction of bilateral lipopolysaccharide (LPS) induced joint inflammation. Seven equines were included in this study (Table 1), six of which were free from lameness and carpal joint disease as judged by clinical exam and radiological screening by two boarded equine specialists (diplomates European College of Veterinary Surgeons (ECVS)), H4 bore a moderate mechanical hindlimb lameness. Animals were stabled individually in single boxes (4 × 4 m) on wood shavings and were familiarised to the environment and routine for two weeks prior to data collection. They were not administered LPS for 14 days prior to this experiment. They were fed concentrate once daily, with regular hay and water provided *ad libitum*. Stables were enclosed in an American style barn where each individual stable had three walls and a front grid bar design allowing them to look out the front of their stable, place their head over the door and maintain observation of the yard and other equines. It was assumed that subjects were exhibiting “normal” behaviour at 0 h and by day 7 of our study, they were approaching or had reached a recovered state (Holcombe et al., 2016).

2.2. Protocol

The left and right dorsal carpal regions of each equine were clipped and prepped for dorsal arthrocentesis. Lipopolysaccharide from *Escherichia coli* O55:B5 (catalogue number L5418; Sigma-Aldrich Ireland Ltd., Arklow, Co. Wicklow Ireland) was diluted to a final concentration of 0.25 ng/mL in sterile lactated Ringer’s solution. Animals were sedated with xylazine (0.2−0.5 mg/kg intravenously, Chanazine 10 %®; Chanelle, Ireland) and butorphanol (0.02−0.04 mg/kg intravenously; Alvegesic vet 10®, ALVETRA u. WERFFT GmbH, Boltzmanngasse 11, A-1090 Vienna, Austria). Arthrocentesis was performed with a 20 G ×40 mm needle and 1 mL LPS solution was delivered aseptically into both left and right intercarpal joints after withdrawal of a synovial fluid sample. Synovial fluid samples for biomarker analyses were obtained at

Table 1
Equine Metadata.

Horse Code	Age	Breed	Sex	Weight (kg)	Height (cm)
H1	12	Con.	G	375	145
H2	17	Con.	M	370	145
H3	16	xCon.	G	341	143
H4	13	Con.	M	344	143
H5	19	ISP	M	400	148
H6	13	UNK	M	406	143
H7	13	UNK	M	336	135

Metadata presented on the 7 horses. A range of Irish type breeds were included: Connemara ponies (Con.), Connemara cross (xConn.), Irish Sports Pony (ISP) and Unknown breeding (UNK). Sex is defined by female/mare (M) and gelding (G).

timepoints: 0, 8, 24, 72 and 168 h. Part of each synovial sampling was placed in EDTA tubes for white blood cell count (WBCC) as an objective indicator of joint inflammation occurring in the model over the timeline of the behaviour analyses. Synovial fluid WBCC were performed manually with the help of a counting chamber under a microscope. Video footage was initiated at the same time for all horses and they were continuously recorded for the first 12 -h period (approximately 8am −8 pm). Animals were recorded again under the same setup at 24, 72- and 168 -hs post LPS for approximately 6−8 hours (approximately 8am −4 pm) Horses were recorded at the same time everyday. A single Hikvision 4 Megapixel EXIR IP PoE turret Camera with 4 mm lens was fixed to the upper right side of each stable, wired out of reach of the animal. This allowed for continuous recording without interfering with normal equine behaviour. Cameras recorded footage throughout the above specified time points to a 4 terabyte Skyhawk CCTV hard drive for download and offline analysis. Videos were sampled at 20 frames/s with a 1280 × 720 resolution. Heart rate and carpal circumference data were recorded at the following time points: 0, 2, 4, 6, 8, 24, 72 &168 h by boarded equine specialists (ECVS). Carpal circumference was measured at the level of the accessory carpal bone with a tape measure. The skin at this point was marked with ink to ensure consistency in measuring technique.

2.3. *Annotation framework*

A bespoke annotation framework was designed through an iterative deductive and inductive analysis of the data. Two research assistants were engaged to work with the principal annotator. Both individuals were students of veterinary medicine, previously experienced in annotating animal footage under the supervision of a consultant animal behaviourist. Expected 'gross' behaviours i.e. eating, drinking, defecating, lying, stepping, etc. were supplemented by a review of existing equine pain literature. Research in laminitic horses or horses presenting with navicular syndrome provide the clinical basis of many bilateral lameness cases, although it was expected that pain experienced by horses under our induced lameness model could be different to that experienced in these conditions. The included behaviours all needed to be detectable by inertial sensor units in order to address our research question.

A modified excel sheet was created for behavioural coding, containing: trial date, session, horse code and joint, food availability, position in the stable, behaviour, actual start time (24 -h clock corresponding to experiment real-time), actual end time, video start time, video end time, time point (e.g. 0 h, 2 h, 4 h, etc.). Drop down menus were instated for behavioural codes, food availability and position in the stable to limit typographical and human error. Formulae were applied to automatically calculate experiment real-time and behaviour duration. A total of 12 behaviours were listed in the original ethogram. The principal annotator randomly chose 4 h of footage from different animals for annotator training and to trial the ethogram. A week later, annotators met and examined the training footage and their separate excel files to highlight any disagreement or misunderstanding of existing operational definitions. Definitions were refined to convey certain behaviours with enhanced specificity; for example, walking activity was split to account for head and neck position, number of consecutive steps and pacing/box walking and ambling activity were considered separately. The literature was consulted a second time to improve the clarity of operational definitions. The ethogram was finalised at 15 behaviours (Table 2). Once the ethogram had been piloted and finalised, video annotation thereafter required >250 h to complete. This supports the findings of Mills and Nankervis (2013) outlining that continuous sampling is usually the most accurate however comes at a significant time investment.

Table 2
Final Ethogram.

Behaviour	Code	Definition
Quiet Standing	QS	The horse has all four hooves in contact with the ground, resting a hindlimb and weight shifts are included. Not intently interested, head drooping, gentle ear flicker or looking or listening with intent/interest, ears fully pricked. Cannot be leaning on another surface or progress away from original position.
Eating	E	Eating hay from the hay rack or concentrate from the feed bowl. Can also be coded as 'E' when food is not available, therefore foraging.
Drinking	D	Drinking from the automatic drinker, coded as soon as the horses places it head in the bowl, ends when head is lifted out.
Lying Down half	LDH	When the horse begins to initiate the getting down action, ending when they return to an upright standing position. Lying on one side with legs flexed towards the body (half recumbency).
Lying down Full	LDF	As above but the horse is lying down flat, on either side, with all four legs splayed (total recumbency).
Rolling	R	When the horse begins to initiate the getting down action, rolling action and ending when they return to the upright standing position.
Resting/holding Forelimb (L)	QSFLL	Pointing, lifting or holding the left forelimb above the ground, not a typical behaviour and typically correlated to serve pain or dysfunction (Ashley et al., 2005).
Resting/holding Forelimb (R)	QSFLR	As above but in the right forelimb.
Pacing	P	Horse is actively walking around the perimeter of the stable with head above withers height. Can include repetitive and back and forth action where the horse moves past the point of origin.
Head over Door	HD	Horse's head is placed over the door, coded from the time it lifts its head over the grid, to when it lifts it back in again.
Urination	U	
Defecation	POO	
Standing Agitation	SA	Not progressive movement, includes: shuffling, tail-swishing, excessive head motion & shaking, frequent weight shifting in the forelimbs (Taylor et al., 2002; Price et al., 2003; Ashley et al., 2005; Wagner, 2010; Gleerup and Lindegaard, 2016).
Ambulation	A	Slow movement around the stable consisting of at least 5 consecutive steps, involving all four legs; horse is moving progressively. Pauses of ≤5 s are included if followed with further stepping (Seaman et al., 2002). Includes walking slowly with neck horizontal or lower, ready to investigate.
Pawing	PAW	Repetitive striking of the ground with a forelimb, associated with weight distribution and [seeking] comfort. Has moderate-good specificity and high sensitivity pain scale (NRS) and a good indicator of orthopaedic pain (Wagner, 2010).

Annotators coded together for approximately 24 h of footage, then began working separately. If an annotator was unsure about coding a given activity, they would share the clip in a private online forum. The activity would be discussed and defined under one of the existing behaviours or deemed unimportant for coding. On completion of each video file, the principal annotator checked each file, randomly selecting behaviour samples to ensure behaviour time points matched the footage and the formulae were calculated correctly in the file. Behavioural coding only began at least 30 min post sedation when the animal returned to full normal behaviour (head above withers, walking normally, interest in food/environment, etc). Sedative effects of a higher dose of xylazine (1.1 mg/kg) are expected to last an average of 20 min (Dugdale, 2011).

The equine data was then stacked in a separate excel file in order of horse (H1 – H7) and session (0–12, 24, 72 and 168 h) to facilitate behavioural modelling, time and frequency analysis.

2.4. *Data processing*

The 15 individual behaviours were collapsed into exploratory sets of similar behaviours. Pain indicators were selected according to existing equine orthopaedic, bilateral limb and hoof pain indicators, i.e. frequent lying episodes and limb pointing or holding indicating severe limb pain (Ashley et al., 2005; Van Loon et al., 2010; Gleerup and Lindegaard, 2016) and pawing as an indicator of orthopaedic pain (Bussières et al., 2008; Wagner, 2010). Standing agitation was initially included in the pain indicator set however it was flagged through preliminary data visualisation and confirmed through video observation that this behaviour is also influenced by food availability. Quiet standing was analysed separately to determine if it indicated an unwillingness to move, as seen in previous pain studies (Reid et al., 2017). Finally, there were 5 categories of behaviours created and analysed and outcome variables were subsequently derived based on these categories of behaviours (Table 3. i.e. Eating, Locomotor Activity, Quiet Standing, Pain Indices, Standing Agitation). Durations of behaviours across time periods were summed and expressed as proportions of time. These proportions were expressed in two-hour blocks to align with sampling periods. Seven separate time periods were created for the purpose of qualitative data visualisation i.e. 0−2 hrs (no LPS effect assumed), 2−4 hrs, 6−8 hrs, 10−12 hrs, 24 h, 72 h and 168 h. LPS injection occurred on day 0 of the experiment. The 24 h, 72 h and 168 hlabels denoted time periods each consisting of 6 h of continuous data collection on days 1, 3 and 7 of the experiment, respectively. Heart Rate, carpal circumference and WBCC were analysed using mean, standard deviation and confidence interval calculations. Five outcome variables were calculated based on behaviours that were grouped into categories as per Table 3. A sixth outcome variable, behaviour variability i.e. the number of behaviour switches per two hours was calculated where behaviour switches were comprised of moving from any of the 15 behaviours to a different behaviour (i.e. frequency of switches). The relationship between the proportion of time spent eating and foraging against the proportion of time that food was available was explored graphically across the experimental time points to understand the impact of food availability.

2.5. Statistical analysis

A one-way repeated measures ANOVA was performed to analyse changes in behaviour across the 7 time points in the following outcome variables: Proportion of time spent eating, Proportion of time spent walking, Proportion of time in quiet standing, Proportion of time in standing agitation, Proportion of time exhibiting pain behaviours and behaviour variability. Mauchly's Test of Sphericity was used to test the assumption of sphericity in each case. Where sphericity was violated, the Huynt-Feldt adjustment is reported. Where a significant effect was detected a contrast, analysis was undertaken to investigate how each condition compared to the first and last conditions i.e. 0 h and 168 h, where horses were assumed to be in their normal/recovered state, or at least approaching this state.

3. Results

White blood cell count peaked at 8 h (Fig. 1), similar to previous reports (De Grauw et al. 2009 a,b, Cokelaere et al. (2018); Sladek et al., 2018) indicating joint inflammation with a suspected association to pain. This was reduced by 24 h–72 h and returned to normal at 168 h (Table 4). A one-way repeated measures ANOVA revealed a significant ($p = 0.046$) overall effect of time. Our assumption is that the horses were experiencing a degree of pain that subsided as the week progressed. Carpal Circumference (Fig. 2) bears a similar trend to mean synovial WBCC, but with no statistically significant differences. Carpal circumference increased 0.85 ± 0.27 cm. Heart rate values remained within normal limits, 39 ± 3 beats per minute. Overall change in heart rate values across the experimental period were 6 ± 2 beats per minute and did not deviate outside of normal range.

Table 3
Behaviours collapsed into categories.

Eating	Locomotor Activity	Quiet Standing	Pain Indicators	Standing Agitation
D	A	QS	LDH	SA
E	P		LDF	
			PAW	
			QSFLR	
			QSFLL	

Behaviour trends are illustrated in Fig. 3 as individual horse data and averaged data. A one-way repeated measures ANOVA was undertaken for each variable. Behaviour variability returned a significant effect of time $p = 0.033$ with contrasts analysis indicating that as the horses recovered, their behavioural variability increased significantly (Table 4).

The proportion of time spent in eating behaviour was plotted against the proportion of time that food was available (Fig. 4). Eating behaviour when food was available appears to have no relationship with time and thus the level of subtle pain experienced here does not appear to influence typical eating behaviours

4. Discussion

Equine behaviour analysis is a prevalent area of research, frequently implemented to better understand behavioural patterns associated with welfare and pain. It was expected that the equines in our study would experience a typical 'inflammation peak' previously associated with induced lameness models. This was exhibited as a spike in WBCC in synovial fluid during 6−12 hrs post LPS injection, returning to normal thereafter. Thus, these equines experienced mild joint inflammation in the latter part of the first 12 -h sampling period that had attenuated by the next day. Our set of specific pain behaviours derived from previous literature (as described in 2.4. data processing) occurred infrequently, with a weak, non-significant trend towards reduced incidence as the week progressed from initial lameness induction. Quantification of walking and quiet standing behaviours were not found to be sensitive markers of bilateral joint inflammation, as large inter-subject differences were noted between horses. Behaviour switching was found to be significantly different during the 6−8, 10−12hr bilateral joint inflammation period, compared to 0 and 168 h i.e. more behaviour switching when the joints were less inflamed. The heart rate data collected in our study also mirrors the trends found in Lucia et al. and supports findings from other pain-behaviour research outlining that physiological measures are not a salient indicator of pain status (Bussières et al., 2008; Gleerup and Lindegaard, 2016; Reid et al., 2017).

Bilateral lameness is an understudied condition with respect to behavioural analysis, thus this study provides a new insight into behaviour in mild bilateral lameness, captured through extensive video annotation. Limitations of this research include that data capture only occurred during daylight hours therefore the horses' diurnal cycle cannot be described; the grouping of behaviours was exploratory and may not be optimal. There was no control group included in the investigation. This is a preliminary investigation of potential behavioural targets for monitoring mild bilateral inflammation and this initial data will inform the continual development of the protocols and technology developed as part of this research programme. In the absence of a control group, we cannot conclude that the behavioural changes observed were due to the intervention, Rather, our data can inform future controlled trials with respect to promising outcome measures that change with inflammation and recovery. There is rarely a control group or 'healthy' reference available to veterinarians on clinical presentation of an animal experiencing pain. Hence it is important to determine if this method can detect between the peak inflammation period, based on clinical biomarkers, and the expected recovery period based on the existing empirical understanding of LPS latency (Bragança et al., 2018)

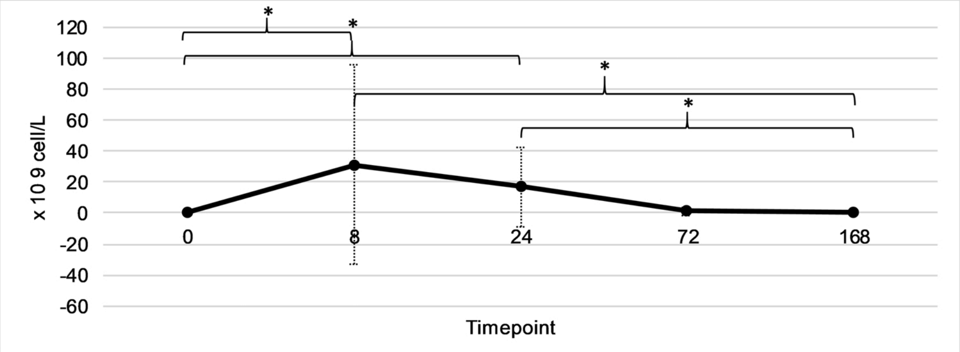

Fig. 1. White Blood Cell Count. Average WBCC vaules plotted with 95 % confidence interval bars. Inflammation peak evident at 8 h following LPS administration. Repeated Measures ANOVA shows a significant overall effect for time (p=0.046). See Table 4 for timepoint contrast analyses.

Table 4
One-way repeated measure ANOVA of behaviours and WBCC.

Outcome Measure	*Effect of Time: p-value*	*Contrasts*
Eating	0.079	NS
Locomotor Activity	0.078	NS
Quiet Standing	0.063	NS
Pain Indices	0.338	NS
Standing Agitation	0.087	NS
Behaviour Variability	0.033*	0 h v 10–12 hrs: p = 0.03 168 h v 10–12 hrs: p = 0.017 168 h v 6–8 hrs: p = 0.018
White blood cell count (x 10 9 cells/L)	0.046*	0 h v 8 h: p = 0.039 0 h v 24 h p = 0.041 8 h vs 168 h p = 0.040 24 h v 168 h p = 0.041

NS = non-significant; Significance denoted by * where $p < 0.05$. Sphericity violated in Locomotor Activity and Standing Agitation analysis.

and an individual horse.

Animals in painful or stressful situations may engage in avoidance, withdrawal behaviours or inactivity (Mellor and Beausoleil, 2015). Therefore, this study investigated durations of active/non-active behaviours and behaviours previously associated with pain following bilateral lameness induction, e.g.locomotor and feeding activity. Locomotor activity and quiet standing, or active/inactive behaviours that are typically measured in actigraphy type approaches did not exhibit statistically significant trends as the experiment progressed. It is interesting to note the large inter-horse variability at 0 h and again at 168 h for locomotor activity (sphericity was violated in this case) and quiet standing, reduced considerably in the 10–12 -h period, behaviour expression across subjects clustered around a reduced value in both cases.

We explored the concept of variability of behaviours and how this might change as the equine moves from high levels of joint inflammation to a return to its normal state. Variability of behaviours i.e., how frequently an animal switches from one behaviour to another has been shown to be an adaptive strategy that denotes an exploratory and thus healthy disposition. Behaviour switching has been investigated to examine equine stereotypies, knowledge acquisition, (Kirsty et al., 2015), anxiety and food related behaviours (Moore-Colyer et al., 2016; Reid et al., 2017). The number of times an animal switched from one behaviour to another thus potentially provides a perspective on how responsive their disposition was to their internal and external environments. Peters et al. (2012) determined that increased behaviour switching of horses in the stabled environment was associated with anticipation behaviours. The authors found that there was increased exploratory behaviour during the anticipation phase of their experiment, suggested to be associated with arousal around reward learning. Behaviour switching may be indicative of more redundancy – or abundance - in the system as levels of pain decreased, if viewed through the principle of motor abundance described in the motor control literature (Latash, 2012). Thus, we interpret behaviour switching in our data as a return to a healthy, adaptive state of being. Behaviour switching appears to be a promising marker of bilateral joint inflammation/pain that could be exploited in future work.

It is important in these types of studies to understand how behaviours interact with each other and/or with environmental and other contextual factors. To this end, we sought to explore how eating behaviours related to food availability, and whether this was influenced by time. Fig. 4 shows that when food was available, the animals usually ate it,

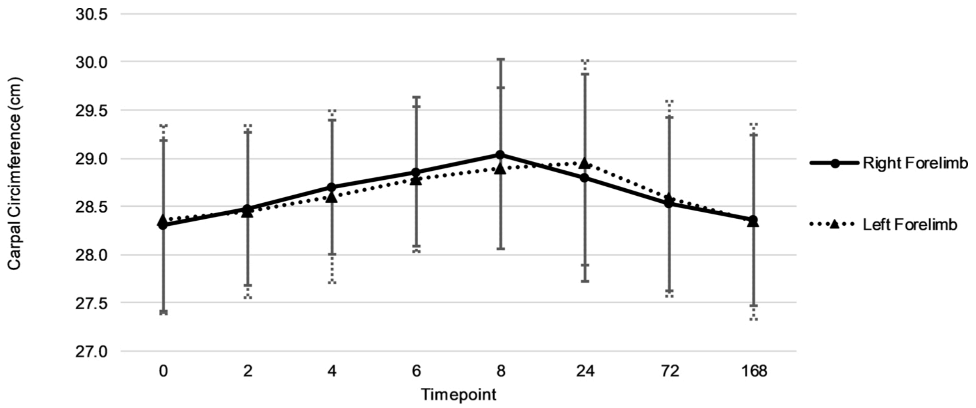

Fig. 2. Carpal Circumference. Average carpal circumference vaules plotted with 95 % confidence interval bars exhibits a peak at 8 h post LPS injection, indicating mild inflammation.

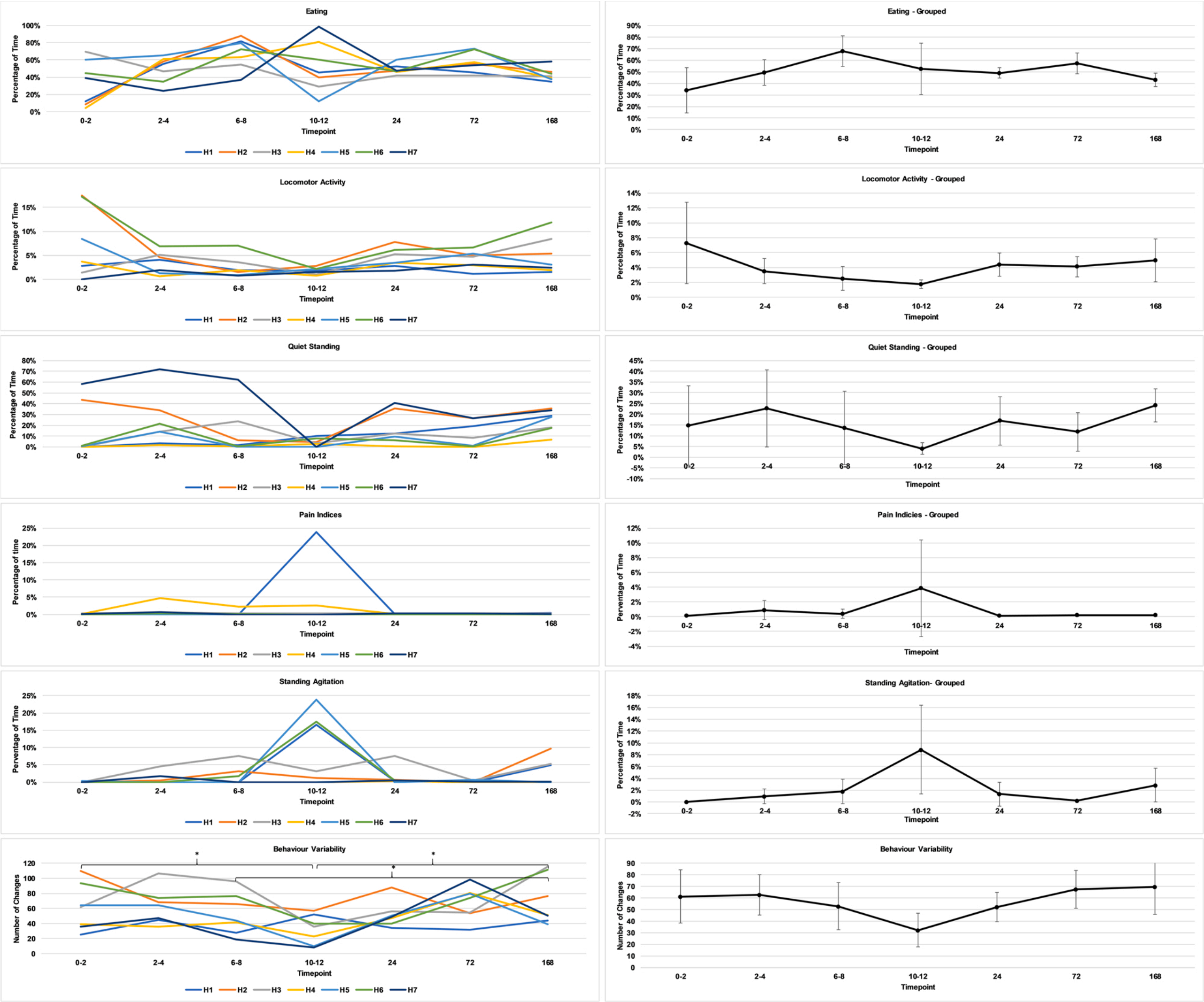

Fig. 3. Behaviour Trends. Collapsed sets of behaviours shown for individual horses and average values plotted with 95 % confidence intervals. Behaviour variability yielded a significant effect indicated by the * $p > 0.05$ with 95 % confidence.

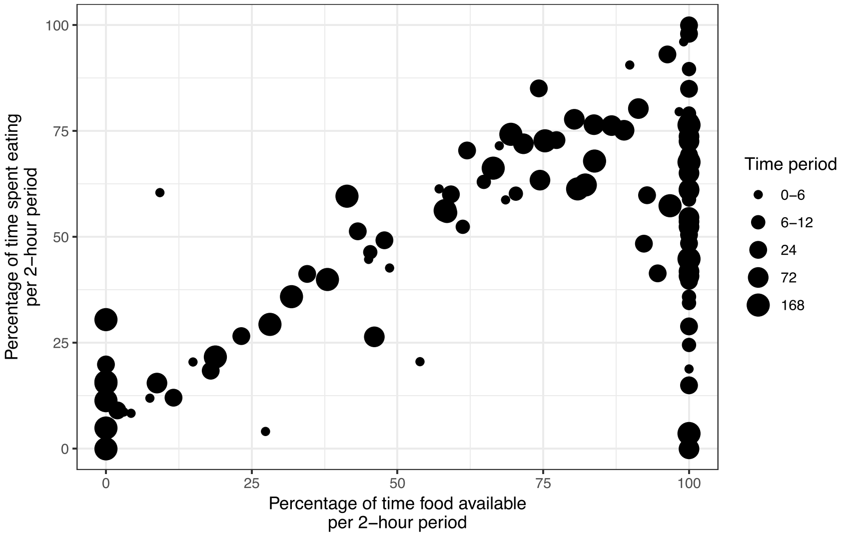

Fig. 4. Food Availability and Eating Behaviour. The proportion of time spent in eating behaviour (which includes foraging) against the proportion of time that food was available, expressed as a function of time since bilateral joint inflammation induction. Eating behaviour when food was available appears to have no relationship with time and thus the level of subtle pain experienced here does not appear to influence typical eating behaviours.

regardless of what stage in the experiment is observed, and was not overtly affected by subtle pain states. The findings illustrated in Fig. 4 suggest that food availability is a contextual factor that may need to be considered when analysing subtle pain behaviours. In this study, forage was administered two to three times daily in a corner hayrack to promote graze style feeding and natural positioning of the animal. Research undertaken by McGreevy et al. (1995) across thoroughbreds in race training yards found that horses which were offered greater amounts of forage more frequently across the day engaged in less abnormal behaviours. They suggested that if the horse's satiety had not been reached, they engaged in greater food searching and oral-based vices (wind sucking/crib biting). Although stabled feeding behaviour is known to be different to pasture style grazing (Heleski et al., 2002), Fig. 4 shows that the equines in this study engaged in food searching behaviour when food was not available. There does not appear to be an effect of time on eating when food was available. Continuous sampling and later annotation of virtually all behaviours was undertaken in this study as it is deemed the most accurate method of behavioural analysis (Mills and Nankervis, 2013). As a proof-of-concept study, we explored the value of longitudinal monitoring of behavioural features, and groups of features alongside mild inflammation and recovery to see if it would be worthwhile to translate such an approach to a wireless, wearable sensor-based system.

Our data, on quantification of locomotor activity, quiet standing and standing agitation highlight the complexity that underpins these behaviours. For example, during the horses' 'inflammation peak' both locomotor activity and quiet standing reduced considerably – uniformly across all horses – while standing agitation increased, but not uniformly. Our analysis of food availability suggests that this contextual factor does influence behaviour at this level of inflammation and may have impacted standing agitation measures. Thus, we conclude that simply quantifying active/non-active behaviour is not an adequate window into bilateral forelimb inflammation on its own.

It is anticipated that the equine behaviours displayed in this study were influenced in some way by human presence and activity. However, it must be considered that there will be a direct/indirect human presence or influence in most scenarios investigating domestic equine behaviours. To mitigate the direct impact of human activity on equine behaviours, anytime a human entered the horses' stable (loose box) and the direct camera view, behaviour sampling was paused and not included in the analysis - it is anticipated the horses' behaviour will have been a direct response to the human presence in the stable space. The duration of time spent in the head over door behaviour (HD), presented in Table 2, may have been influenced by the activity in the stable yard. However, it is not possible to state that this was because of human activity, as no behaviour analysis was completed in the absolute absence of human presence. There were many occasions, particularly from timepoint 24 h onwards when the stable yard was very quiet with minimal activity and the horses still engaged in HD behaviour.

Distinguishing parameters of "normal" and "abnormal" activity in equines has proven challenging. Several behavioural monitoring methods have been employed, many of which focus on interval based focal or scan sampling (de Grauw and van Loon, 2016). Focal sampling is defined as noting all behaviours and physical features occurring within a given time frame (Altmann, 1974); for example, five minutes per horse in two-hour windows. Focal and scan sampling styles are used to reduce the time required to assess pain/behaviour. However, it can be argued based on our results that this method is insufficient when pain is not severe and pain indicators like those tracked in our study, such as pawing, occur infrequently. Given the amount of time that was required to monitor these equines using video annotation (>250 h in total), continuous monitoring is clearly not a feasible solution to the issue of potentially missing important, infrequent behaviours if using focal sampling. Indeed, it has previously been reported to be a time consuming and inefficient method of pain detection (de Grauw and van Loon, 2016). In addition to practical monitoring challenges, our results have shown an important influence of individual difference across equines for active versus inactive behaviours. This individual difference has been also demonstrated by previous authors. McDuffee et al. (2000) used wearable technology to monitor limb loading in 5 normal horses and one with a repaired metatarsal fracture over a 24 h period, they found that loading rates were hugely variable across normal horses. Holcombe et al. (2016) investigated the residual impact of repeated application of 30 ng/kg LPS. The authors captured biomarkers and pain behaviours. They found that each horse expressed a level of pain but alluded to large inter-individual but consistent intra-individual behaviours; i.e. each horse would express their own suite of pain-behaviours each time. Researchers have emphasised the importance of knowing what is normal or abnormal on an individual horse-by-horse basis (Mills and Nankervis, 2013). This is particularly relevant when clinicians are investigating a horse's health status, where owner knowledge is an important aspect of the clinical assessment as it provides the benchmark of what is normal or abnormal for the individual horse (McGreevy, 2012). Thus establishing "normal" and "abnormal" thresholds for the "average horse" is somewhat artificial and would be of questionable value in the applied setting.

A possible solution to the above challenges would be the application of wearable sensor devices for longitudinal, remote monitoring of individual horses. These technologies have been investigated in equine research to reduce the human subjectivity of assessment and the time required to monitor individual activity patterns (Coleman et al., 1999; Burla et al., 2014). Automatic behaviour detection using sensor technology is a frontier that is primed for exploitation in equine clinical and sporting settings. Lloyd Morgan's canon states that we should not seek to explain behaviour in terms of complicated physiological processes if a simple explanation will do (2013). Behaviour switching may represent a holistic measure of adaptive behaviour which could provide this simplicity. McGreevy et al. (2012) acknowledge that technological advances are likely to improve education. Investigation into behaviour variability, through actigraphy-type methodologies, opens the opportunity to automate longitudinal behaviour monitoring. We conclude from this exploratory study that the analysis of gross behaviours - as captured using the ethogram developed for this study - may be valuable in the detection of subtle bilateral inflammation in equines – a condition that is difficult to discern subjectively. Future work will seek to optimise groupings of behaviours associated with subtle levels of inflammation in a larger sample and different pain presentations using wearable technology. Existing pain-behaviour grimace and composite scales have been validated and used accurately to assess pain threshold in horses. The potential for automation of such scales exist through face recognition technology, however this technology tends to malfunction if the face it identifies is distorted or partially concealed. Gross behaviours augmented with in-depth analysis of sensor signals e.g. accelerometer, gyroscope or load based signals would provide further insights into the quality of movement.

Future work will refine this behavioural model and investigate its repeatability. Approaches to develop more individualised profiling could incorporate kinematic data and further indices of variability to enhance the power of the model in detecting subtle levels of bilateral lameness/pain. Severe and unilateral lameness/pain can be easily discerned by the naked eye. The value of identifying subtle levels of bilateral lameness/pain lies in the opportunity for early intervention and resolution of major equine health issues such as laminitis, navicular disease and osteoarthritis that often have a multi limb component. This could be possible through the objective, remote analysis of hours of stable-based behaviour data that would otherwise go unseen.

Declaration of Competing Interest

The authors report no declarations of interest.

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