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Development of a composite orthopaedic pain scale in horses ☆

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Abstract

This study addresses development and validation of a composite multifactorial pain scale (CPS) in an experimental equine model of acute orthopaedic pain. Eighteen horses were allocated to control (sedation with/without epidural analgesia – mixture of morphine, ropivacaine, detomidine and ketamine) and experimental groups: amphotericin-B injection in the tarsocrural joint induced pain and analgesia was either i.v. phenylbutazone administered post-induction of synovitis, or pre-emptive epidural mixture, or a pre-emptive combination of the 2. Inter- and intra-observer reproducibility was good ($0.8 < K < 1$). The key specific and sensitive behavioural indices were response to palpation of the painful area, posture, and, of lesser value, pawing on the floor, kicking at abdomen and head movement. Of particular interest was the statistical correlation observed between the CPS and both non-invasive blood pressure ($P < 0.0001$) and blood cortisol ($P < 0.002$). This study established the value of some behavioural and physiological criteria in determining equine orthopaedic pain intensity and clearly demonstrated that pre-emptive, multimodal analgesia provided better management than the two other protocols tested.

Keywords: Horse; Acute orthopaedic pain; Experimental model; Behaviour; Physiological parameters; Validation

1. Introduction

Pain is a complex sensory experience normally generated by the activation of high-threshold receptors (nociceptors). Nociceptors send electrical signals from periphery to spinal cord and brain, producing responses that warn and protect the host from impending tissue damage, thereby helping to maintain bodily integrity and survival (Craig, 2003; Muir, 2005).

The evaluation of pain severity is particularly important for clinical decision-making. Direct measurement of a subjective experience is not possible; therefore pain assessment is a value judgment relying on behavioural and physiological indices to provide indirect evidence of mental state (Hansen, 1997; Molony and Kent, 1997). Pain assessment should involve all of its dimensions, including intensity, frequency, duration and quality, although this is not an exhaustive list (Ashley et al., 2005).

Pain experience and expression are influenced by many factors such as species, breed and individual variations, environmental characteristics, drugs (Flecknell, 2000a). For instance, the instinctive response of horses to aversive stimuli is "flight". However, once confined, the only possible response may be an aggressive behavioural attack at the

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pain source or threat (Casey, 2004). Aggression has been strongly associated with pain in horses (Ashley et al., 2005), as a genuine pain response to palpation, as a fear response in anticipation of the pain-related stimulus, or through learned association, such as linking their own offspring with parturition pain (Juarbe-Diaz et al., 1998). This can make the interpretation of their behaviour more difficult, not being clearly able to differentiate true pain from an unpleasant situation (Flecknell, 2000b). There is a paucity of scientific research on equine pain behaviour despite the clinical importance placed on a horse's demeanour.

As highlighted in a recent review (Ashley et al., 2005), an ideal pain scoring system needs to be linear, weighted, sensitive to pain-type, breed- and species-specific, less dependent on the observer and closed to misinterpretation. However, few methods of pain evaluation/quantification have been developed in horses, and to our knowledge, none of them include physiological and behavioural responses as well as responses to treatment parameters (Table 1). The accent has been placed on metabolic and hormonal changes associated with peri-operative pain (Robertson, 1987; Robertson et al., 1990; McCarthy et al., 1993; Raekallio et al., 1997; Hubbell, 1999; Price et al., 2003). Only a few studies have evaluated behavioural indicators (Raekallio et al., 1997; Hubbell, 1999; Goodrich et al., 2002; Price et al., 2003; Pritchett et al., 2003; Rietmann et al., 2004). These authors used subjective numerical rating scales (discontinuous, ordinal scale) with (Goodrich et al., 2002; Pritchett et al., 2003) or without (Hubbell, 1999; Pritchett et al., 2003) video recordings, composite multifactorial scales (complex and multidimensional elements) (Raekallio et al., 1997) or automated video-analysis (Price et al., 2003; Rietmann et al., 2004). Mainly based on the own clinical experience of the authors, such scales could not be extended to other practices due to lack of validation, and elevated costs/time consumption of automated video-analysis.

To be an efficient pain evaluation tool, a pain scale must be easy to use, include parameters giving repeatable interpretation from one evaluator to another, and provide constancy in the results obtained. Parameters are also required to give an evaluation specific to the presence or absence of pain and allow the observer to differentiate as precisely as possible the degree of pain encountered by the patient despite the absence of verbal communication with the animal. The purpose of this study was to complete the validation of a composite pain scale (CPS), based on physiological and behavioural criteria, by determining if it could be (1) repeatable, specific and sensitive on an inflammatory, orthopaedic, experimental equine model; and (2) suitably applied to evaluate the quantitative and qualitative effects of analgesic therapy in the same experimental setting. To attain these objectives this study compares non-painful appropriate controls with chemically-induced acute articular pain groups submitted to monotherapeutic, post-injury or multimodal, pre-emptive analgesic protocols.

2. Materials and methods

The study protocol was approved by the Ethics Committee (#03–09) of the École Nationale Vétérinaire de Lyon.

We favoured a rigorous scientific approach based on standardized operating procedures (SOP) to (1) use the CPS (assuring repeatable intra- and inter-observer assessment) and (2) assure the homogeneity of our experimental model: similar horses were selected, procedures were standardized to be repeated in the same sequence and at the same time of the day, with pain induction method identical in all horses.

2.1. Horses

Eighteen standard-bred horses (10 mares, 8 geldings), 5–10 years old (median 6.9 years) and weighing from 450 to 530 kg (median 488 kg), were included in the study. The horses were deemed healthy after physical examination and behavioural evaluation.

2.2. CPS

The pain scale developed in this study is a modification of existing scales (for review, see Ashley et al., 2005). It is a multifactorial numerical rating CPS incorporating physiologic, response to treatment and behavioural data which were believed to best identify orthopaedic pain (Table 1). All measurements were rated from 0 to 3, with zero corresponding to normality and no modification in the absence of pain, and three corresponding to the most significant modification in the presence of pain. The maximum total pain score (total CPS) that can be achieved with this scale is 39.

2.3. Study design

All horses were randomly assigned to six groups, each containing three horses. Three control groups, without pain induction, were used to verify the specificity of the CPS parameters to pain, whereas three experimental groups, in which we induced synovitis pain, served to assess the sensitivity of the CPS parameters to pain: the same model of inflammatory synovitis pain was induced in each of nine horses, and these were further subdivided into groups of three, each of which received different analgesics in order to produce different levels of pain. The study was double-blinded for external video observers whereas the real-time assessor was blinded inside control and experimental groups.

The pain model in this study was a chemically-induced synovitis by injection of amphotericin-B (Fungisone®, 50 mg, Bristol–Myers Squibb, Paris, France) in a single tarsocrural joint (Peloso et al., 1993; Sysel et al., 1996).

The negative control groups were defined as follows:

- C_0: sedation only.
- C_1: sedation and epidural placebo.
- C_2: sedation and epidural analgesia.

Table 1
Multifactorial numerical rating composite pain scale (CPS)

Physiologic data	Criteria	Score/12
Heart rate	Normal compared to initial value (increase <10%)	0
	11–30% increase	1
	31–50% increase	2
	>50% increase	3
Respiratory rate	Normal compared to initial value (increase <10%)	0
	11–30% increase	1
	31–50% increase	2
	>50% increase	3
Digestive sounds (bowel movements)	Normal motility	0
	Decreased motility	1
	No motility	2
	Hypermotility	3
Rectal temperature	Normal compared to initial value (variation < 0.5 °C)	0
	Variation les 1 °C	1
	Variation les 1,5 °C	2
	Variation ges 2 °C	3
Response to treatment	**Criteria**	**Score/06**
Interactive behaviour	Pays attention to people	0
	Exaggerated response to auditory stimulus	1
	Excessive-to-aggressive response to auditory stimulus	2
	Stupor, prostration, no response to auditory stimulus	3
Response to palpation of the painful area	No reaction to palpation	0
	Mild reaction to palpation	1
	Resistance to palpation	2
	Violent reaction to palpation	3
Behaviour	**Criteria**	**Score/21**
Appearance (reluctance to move, restlessness, agitation and anxiety)	Bright, lowered head and ears, no reluctance to move	0
	Bright and alert, occasional head movements, no reluctance to move	1
	Restlessness, pricked up ears, abnormal facial expressions, dilated pupils	2
	Excited, continuous body movements, abnormal facial expression	3
Sweating	No obvious signs of sweat	0
	Damp to the touch	1
	Wet to the touch, beads of sweat are apparent over the horse's body	2
	Excessive sweating, beads of water running off the animal	3
Behaviour	**Criteria**	**Score**
Kicking at abdomen	Quietly standing, no kicking	0
	Occasional kicking at abdomen (1–2 times/5 min)	1
	Frequent kicking at abdomen (3–4 times/5 min)	2
	Excessive kicking at abdomen (>5 times/5 min), intermittent attempts to lie down and roll	3
Pawing on the floor (pointing, hanging limbs)	Quietly standing, no pawing	0
	Occasional pawing (1–2 times/5 min)	1
	Frequent pawing (3–4 times/5 min)	2
	Excessive pawing (>5 times/5 min)	3
Posture (weight distribution, comfort)	Stands quietly, normal walk	0
	Occasional weight shift, slight muscle tremors	1
	Non-weight bearing, abnormal weight distribution	2
	Analgesic posture (attempts to urinate), prostration, muscle tremors)	3
Head movement	No evidence of discomfort, head straight ahead for the most part	0
	Intermittent head movements laterally or vertically, occasional looking at flanks (1–2 times/5 min), lip curling (1–2 times/5 min)	1
	Intermittent and rapid head movements laterally or vertically, frequent looking at flank (3–4 times/5 min), lip curling (3–4 times/5 min)	2
	Continuous head movements, excessively looking at flank (>5 times/5 min), lip curling (>5 times/5 min)	3

Table 1 (*continued*)

Behaviour	Criteria	Score
Appetite	Eats hay readily	0
	Hesitates to eat hay	1
	Shows little interest in hay, eats very little or takes hay in mouth but does not chew or swallow	2
	Neither shows interest in nor eats hay	3
Total CPS		39

The positive experimental groups were all pain-induced and were distributed as follows:

- E_0: sedation and post-injury rescue i.v. non-steroidal anti-inflammatory drug (NSAID) monotherapy.
- E_1: sedation and pre-emptive epidural analgesia.
- E_2: sedation and pre-emptive i.v. NSAID with epidural analgesia.

2.4. Procedures (Fig. 1)

The day of the experiment all CPS data were recorded prior to sedation. At T_{-2}, the horses were sedated with acepromazine (0.03 mg/kg bwt i.v., Vétranquil®, 1%, Sanofi Santé Nutrition Animale, Libourne, France) to facilitate sterile placement of a 14-G catheter (Angiocath®, Becton Dickinson Infusion Therapy Systems Inc., Sandy, UT, USA) in one of the jugular veins, 10 min later, and then returned to a quiet area for the next 20 min. Afterward, all the horses were moved into stocks, and epidural catheters (Perifix®, B. Braun Medical Division, Melsungen, Germany) were placed aseptically in the first coccygeal interspace with a 10-cm length put in the epidural space (Lainay, 2001). At T_{-1}, horses were sedated with romifidine (0.04 mg/kg bwt i.v., Sédivet®,0.876%, Boehringer Ingelheim, Paris, France). Sedation and physiological parameters were evaluated at $T_{-0.75}$, according to a numerical rating scale following a linear progression, with subjective interpretation of descriptors (absent = 3, light = 2, moderate = 1 and heavy = 0 sedation).

At $T_{-0.5}$, horses belonging to groups C_2, E_1 and E_2 were administered an analgesic mixture through the epidural catheter. It combined ropivacaine (0.15 mg/kg bwt, Naropéïne®, 1%, Laboratoire Astra France, Groupe pharmaceutique Astra Suède, Nanterre, France), detomidine (0.02 mg/kg bwt, Domosedan®, 1%, Pfizer Santé Animale, Orsay, France), morphine (0.05 mg/kg bwt, Morphine Lavoisier®, 1%, Laboratoire Chaix et Du Marais, Paris, France), and ketamine (0.50 mg/kg bwt, Imalgene 1000®, 10%, Mérial SAS, Lyon, France), completed to a total volume of 0.023 mL/kg bwt with sterile physiological saline. The safety and analgesic efficacy of this epidural solution were tested in a previous study (Lainay, 2001). Horses belonging to groups C_1 and E_0 were epidurally administered the same total volume of sterile saline as a placebo. Horses from group C_0 underwent epidural catheter placement without injection. Only group E_2 received a pre-emptive injection of phenylbutazone (2.20 mg/kg bwt i.v. BID, Phenylarthrite®, 2%, Laboratoire Vétoquinol SA, Lure, France) that was repeated at T_{12}. Horses from all other groups received a placebo i.v. injection of sterile physiological saline. At $T_{-0.25}$, *E-groups* horses were injected with 25 mg of amphotericin-B in 5 mL of sterile water in the left tarsocrural joint.

For all groups, experimental follow-up began at T_0. It was performed over a 24-h period with evaluation:

✓ Every hour up to T_{12} and every 6 h up to T_{24} for the *C-groups*.
✓ Every hour up to T_{24} for the *E-groups*.

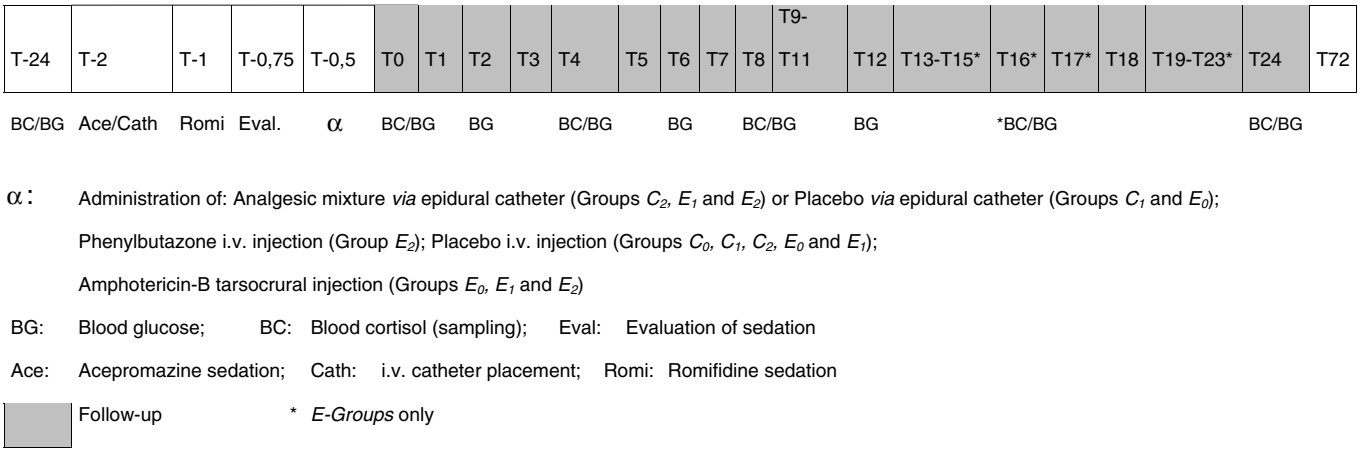

Fig. 1. Time line of events.

✓ Horses from the *E*- and *C-groups* were euthanized at T_{24}, and T_{72} respectively. At this time, the horses received sedation with xylazine (1.1 mg/kg bwt i.v., Rompun®, 2%, Bayer Pharma, Division Santé Animale, Puteaux, France) followed by injection of an embutramide and mebezonium iodide solution (100 mL per horse i.v., T61®, Hoechst Roussel Vet, Pantin, France).

For each time-point, the real-time blinded assessor (C.J.):

✓ Made two short video recordings with a video digital camcorder (2–8 min), first while the horse was left undisturbed in the box (and unaware of the video realization) and, second, while the horse underwent palpation of the tarsocrural area.
✓ Scored each horse for signs of pain using the CPS (Table 1).
✓ Recorded indirect, non-invasive systemic arterial blood pressure (NIBP, diastolic, mean, and systolic) with an oscillometric device (Siemens Sirecust 402, Normed, St-Thibeault des Vignes, France) following a SOP standardizing the cuff size selection as 40% circumference of the tail base, where the cuff was systematically placed. The SOP has been previously validated, while comparing the NIBP monitorig with a direct measure of systemic arterial blood pressure at the level of the facial transverse artery (Lainay, 2001).
✓ The video recordings were viewed by two external assessors (JLC, ET) with experience in scoring animal behaviour, to assess each horse in the response to treatment and behavioural categories of the CPS.

Blood samples were obtained from the jugular vein. For cortisol analysis, 3 mL of blood was collected in heparinized tubes at $T_{-24,0,4,8,}$ and $_{24}$ for all *C-groups* and same sample times plus an additional one at T_{16} for all *E-groups*. Blood was then centrifuged at $1{,}500 \times g$ for 10 min. Plasma was collected and frozen at −20 °C for 1–4 h and at −70 °C until further analysis (1 month later) of cortisol by radioimmunoassay (Amerlex RIA, Ortho-Clinical, Issy-Les-Moulineaux, France). Glucose was measured in whole blood with a glucometer (Glucotrend, Roche Diagnostics, Meylan, France) at $T_{-24,0,2,4,6,8,12}$ and $_{24}$ for the *C-groups* and same sample times plus an additional one at T_{16} for the *E-groups*.

2.5. Administration of analgesics

The administration of rescue analgesia in the *E-groups* was based on the total CPS score reached by the horse during its evaluation as a function of time. When the total CPS score reached a moderate level of pain, such as CPS = 13/39 (third of the total), horses received an epidural injection of saline-placebo in group E_0 (no analgesia) or of analgesic mixture in groups E_1 and E_2 to ensure better maintenance of the induced pre-emptive analgesia (Fig. 2). When the total CPS score reached 20/39 (half of the total), horses of any group received an injection of phenylbutazone (2.20 mg/kg bwt i.v. BID) for ethical purposes to prevent a higher level of pain (Fig. 2). Different evolutions with time on the CPS, amount of rescue analgesia needed and time required between injections indicated the efficacy of analgesic treatment in each group and allowed the classification of three different pain levels. To assure blindness of the study (in comparison to group E_2), horses in group E_0 received an i.v. injection of sterile physiological saline at T_0 and T_{12} and an epidural placebo when CPS $\geqslant$ 13/39. In the same manner, horses from group E_1 received an i.v. injection of sterile physiological saline at T_0 and T_{12}.

2.6. Statistical analysis

We restricted CPS comparison between groups to the period where only a limited number of horses had received real (saline epidural injection in group E_0 was not considered – see Fig. 2) rescue analgesia, i.e., up to T_7. Total CPS scores and individual parameter scores were analyzed for all 18 horses. Statistical analysis focused on five objectives:

2.6.1. Inter- and intra-observer reproducibility (reliability)

The *K*-coefficient of agreement was used to determine the degree of reproducibility of measurements among the three assessors (inter-observer repeatability) for their evaluation of each parameter in the response to treatment and behavioural categories ($n = 9$ parameters). The *K*-coefficient for each pair of assessors was calculated with a contingency table.

Intra-group values obtained for groups C_0 and C_1 at $T_{8,12,18}$, and $_{24}$ were compared by *K*-coefficient of agreement to assess intra-observer reproducibility for the three observers.

2.6.2. CPS specificity and precision (validity)

The percentage of expression (considered as any value different from 0) of a parameter in the *C-groups* determines its specificity to the presence of pain; the lower the percentage of expression, the more specific is a parameter. If fewer

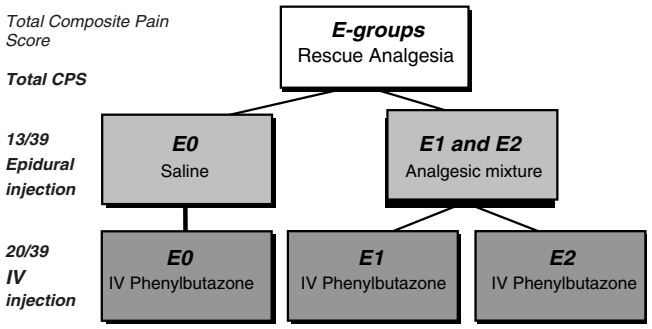

Fig. 2. Methodology of rescue analgesia use.

than 5% of control horses' values exhibited for example kicking, this behaviour was then stated as having excellent specificity. As guidelines, we selected the following percentages:

- 0–4.9%: Excellent specificity.
- 5–14.9%: Good specificity.
- 15–29.9%: Moderate specificity.
- $\geqslant$30%: No specificity.

This occurrence rate was judged in each *C-group* (based on 45 values) and also relatively to the results get for the nine horses, representing 135 values.

2.6.3. CPS sensitivity (responsiveness)

We first needed to verify that the responsiveness of the CPS with time was different for each *E-group*, allowing to distinguish three pain levels perceived from the lowest to the highest level by horses where $E_2 < E_1 < E_0$ intensity of pain.

Secondly, the sensitivity of each parameter was determined in each *E-group* by paired comparison between two different *E-groups* using odds ratio; a parameter was considered as highly sensitive if it could distinguish between the three *E-groups*, as moderately sensitive when 1 *E-group* distinguished itself from the two others, and as not sensitive if it was unable to distinguish any *E-group*.

2.6.4. Complementary physiological criteria

Mean NIBP, cortisol and glucose were comparative physiological parameters.

All continuous dependent variables were analyzed with a mixed linear model for repeated measures with time (intra-subject factor), and group (between-subject factor), using SAS software (SAS version 9.0, Cary, NC, USA). A similar model was tested for total CPS scores since most horses showed at least five distinct score values. A multinomial logistic regression model for specific variables measured on an ordinal scale was adopted. Significance level was set at 0.05 throughout. Results are presented as mean $\pm$ standard deviation. *A priori* contrasts were performed to compare different levels of the independent variables at different time-points.

2.6.5. Analgesia efficacy

The total amount of epidural and i.v. injections was considered as a primary endpoint and compared between all *E-groups*. We also considered for analysis the latency to first use of rescue analgesia, as well as the interval of time required between two successive rescue analgesia injections. Non-parametric tests were used to analyze these parameters because of small sample size and non-Gaussian distribution. The *E-groups* were compared by the Kruskal–Wallis test with pair-wise post hoc contrasts. Significance level was also set at 0.05, and data presented as mean $\pm$ standard deviation.

3. Results

Romifidine sedation showed minimal differences between groups: at $T_{-0.75}$ the mean score was 0.89 ± 0.6, and 1.22 ± 0.8, in *C-* and *E-groups*, respectively ($P = 0.68$). Physiological parameters changes caused by romifidine sedation were minimal (diminution of heart rate, respiratory rate, digestive sounds and temperature) and normalized at T_0 (heart rate, respiratory rate, digestive sounds) or T_1 (temperature).

Results on reproducibility, specificity and sensitivity are summarized in Table 2.

3.1. CPS (total and individual parameter) reproducibility

High inter-observer repeatability was obtained, with all K values ranging between 0.8 and 1 for each of the response to treatment or behavioural parameters. Consequently, the unique values used for subsequent analyses were values obtained by the real-time observer (C.J.). There was also high intra-observer reproducibility in the values obtained for each observer when comparing groups C_0 and C_1 at times $T_{8,12,18}$ and $_{24}$.

3.2. Total CPS specificity and sensitivity (Fig. 3)

Time ($P = 0.0003$) and group ($P < 0.0001$) influenced the total CPS. In the *C-groups*, for total CPS, no significant difference ($P > 0.15$) was evident between the three groups, indicating no effect of the epidural injection (either saline = C_1, or analgesic mixture = C_2) on the CPS, and, globally, the specificity of the CPS to pain was good.

A priori contrasts showed that the *E-groups* were all different from the *C-groups* except for groups E_2 and C_2 where no difference ($P > 0.09$) was observed at any time-point. Between the *E-groups*, CPS values in group E_0 were significantly higher than in groups E_1 ($P < 0.03$) and E_2 ($P < 0.01$) from T_2 to T_7. A statistically significant ($P = 0.002$) difference was also observed between groups E_2 and E_1 at T_6, and the difference was marginally non-significant at T_4 and T_5 ($0.05 < P < 0.07$). The total CPS was able to distinguish three pain levels in the *E-groups*, but the presence of only one significant time-point between groups E_2 and E_1 did not allow us to classify the sensitivity of the CPS as excellent.

3.3. Individual parameters specificity and sensitivity

3.3.1. Physiological responses

Heart rate specificity was considered moderate: it showed expression values different from 0 in groups C_0 (21.5%), C_1 (11.1%), and C_2 (5.7%). Heart rate was classified as having moderate sensitivity to pain: Group E_0 values were significantly different from group E_1 ($P < 0.0001$) and group E_2 ($P = 0.003$). However, odds were similar between groups E_1 and E_2 ($P = 0.33$).

Table 2
Summary of evaluation of reproducibility, specificity and sensitivity for the CPS and individual parameters of the CPS as well as complementary physiological criteria

Total CPS	Reproducibility[a]	Specificity[b]	Sensitivity[c]	Odds ratio for sensitivity[d] E_0 vs. E_1/E_0 vs. $E_2//E_1$ vs. E_2
	Good	Good	Good-to-excellent	
Individual parameters				
Physiological parameters				
Heart rate		Moderate	Moderate	29.4 ($P < 0.0001$)/13.8 ($P = 0.003$)//$P = 0.33$
Respiratory rate		Moderate	Moderate	112 ($P = 0.0003$)/118 ($P < 0.0001$)//$P = 0.97$
Digestive sounds		Good-to-moderate	Null	No analysis
Rectal temperature		Null	Null to moderate	$P = 0.35$/$P = 0.18$//0.067 ($P = 0.01$)
Behavioural parameters				
Appearance	Good	Null	Moderate	43.7 ($P < 0.0001$)/33.4 ($P = 0.0003$)//$P = 0.32$
Posture	Good-to-excellent	Excellent-to-good	Excellent	3.7 ($P = 0.04$)/38.7 ($P = 0.0003$)//12.5 ($P = 0.009$)
Sweating	Excellent	Good	Null	No analysis
Head movement	Good-to-excellent	Moderate-to-null	Excellent	3.4 ($P = 0.009$)/29.3 ($P = 0.0004$)//11.7 ($P = 0.0047$)
Kicking at abdomen	Excellent	Excellent	Excellent[e]	No analysis
Appetite	Good-to-excellent	Good	Null to moderate	$P = 0.12$/4.2 ($P = 0.015$)//$P = 0.84$
Pawing on the floor	Good	Good-to-moderate	Excellent	4.8 ($P = 0.0002$)/66.8 ($P < 0.0001$)//13.8 ($P = 0.003$)
Response to care				
Interactive behaviour	Excellent	Excellent-to-good	Null to moderate	No analysis
Response to palpation of the painful area	Good-to-excellent	Excellent	Excellent	2.7 ($P = 0.002$)/9.3 ($P < 0.0001$)//3.4 ($P = 0.0003$)
Complementary physiological criteria				
Mean systemic arterial blood pressure		Good	Excellent	
Blood glucose		Null	Null	
Blood cortisol		Good	Moderate	

[a] Inter-observer reproducibility was tested with the *K*-coefficient of agreement and was considered as excellent with $1 < K < 0.9$, and good with $0.9 < K < 0.8$.

[b] Specificity was tested with the percentage of occurrence of the parameter in the *C-groups* as different from 0. With an occurrence of 0–4.9%, specificity was considered as excellent, 5–14.9% as good, 15–29.9% as moderate, $\geqslant 30\%$ as null.

[c] Sensitivity was tested with the possibility of distinguishing between the *E-groups*. If the parameter distinguished the three groups from each other, then sensitivity was considered as excellent; only one group from the two others, then sensitivity was considered as moderate; and not distinguishing any group, then sensitivity was considered as null.

[d] Odd ratio was calculated for sensitivity when comparing *E-groups* one to another. For each comparison (when statistically possible), the odds ratio is presented as well as the *P*-value of the comparison. No analysis was done when the prevalence of the parameter in each group was too low (see text for more details).

[e] Is present when weak prevalence of the parameter did not allow statistical confirmation of descriptive data.

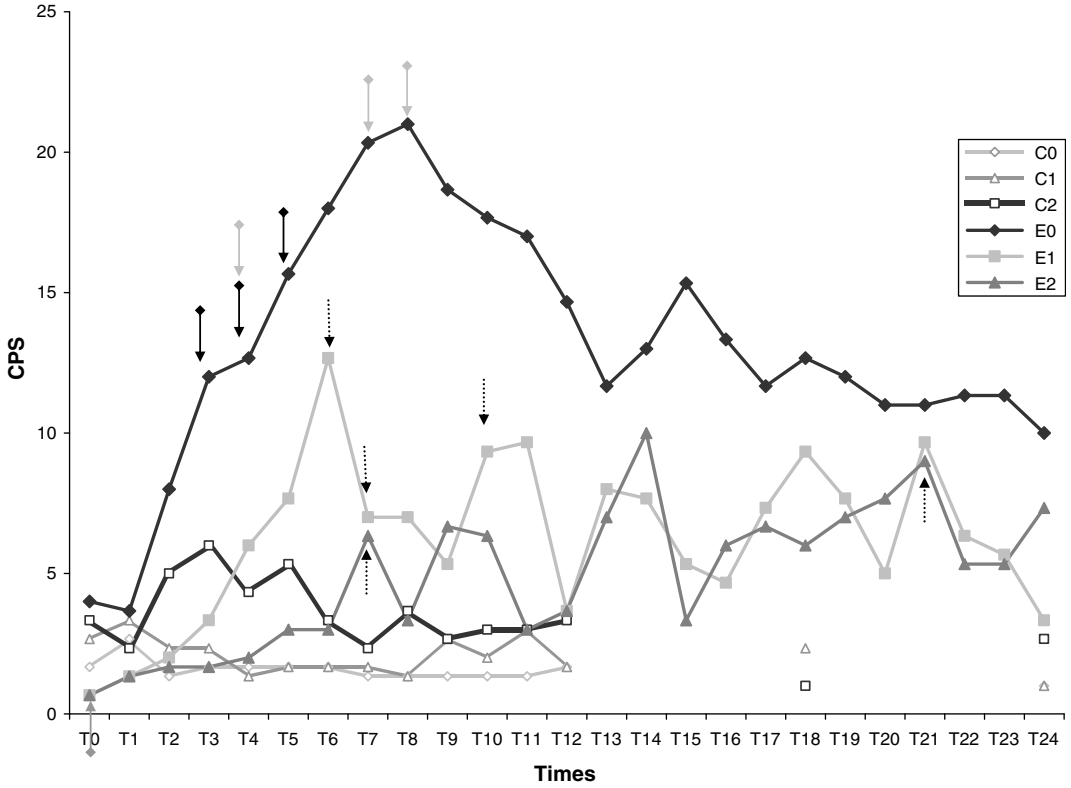

Fig. 3. Evolution with time of the total composite pain scale (CPS). The control groups (*C-groups*) were followed hourly from T_0 to T_{12} and then at T_{18} and T_{24}. C_0 = sedation + epidural sham; C_1 = sedation + epidural saline; C_2 = sedation + epidural analgesics. The experimental groups (*E-groups*) were followed hourly during the whole 24-h period. E_0 = rescue i.v. phenylbutazone + epidural saline; E_1 = pre-emptive i.v. saline + epidural analgesics; E_2 = pre-emptive i.v. phenylbutazone + epidural analgesics. The grey arrows with a diamond indicate, for each horse, the first injection of phenylbutazone i.v., either rescue (head down, group E_0, when CPS $\geqslant$ 20/39) or pre-emptive (head up, group E_2). The black arrows with a diamond indicate, for each horse, the first rescue injection of epidural saline (Group E_0, when CPS $\geqslant$ 13/39). The shaded arrows indicate, for each horse, the first rescue injection of epidural analgesics (head down, group E_1; head up, group E_2) required when CPS $\geqslant$ 13/39.

Respiratory rate presented moderate specificity with modification of 11.1% in group C_0, 5.7% in group C_1, and 17.5% in group C_2, the epidural analgesic mixture inducing a mild increase in the respiratory rate. Respiratory rate was classified as having moderate sensitivity to pain.

Digestive sounds were deemed to have good-to-moderate specificity, with values strongly modified from normality in group C_2 (48.2% during the first 8 h, and 15.8% for the whole period), and no effect in groups C_0 and C_1, which suggests an effect of the epidural analgesic mixture on gastrointestinal transit/motility. This parameter was not sensitive (weak prevalence of 7.3% in group E_0 alone; no analysis done).

Rectal temperature was not considered specific to pain as it was highly responsive in groups C_0 (25.9%), C_1 (40.7%) and C_2 (48.2%). It was also not a sensitive parameter with similar odds between groups, except that they decreased by a factor of 15 in group E_1 compared to E_2 ($P = 0.01$).

3.3.2. Behavioural responses

Appearance was non-specific, with very high modification from what was considered normal behaviour. All 3 *C-groups* showed high expression of the parameter: Group C_0, 92.6%; C_1, 92.6%; and C_2, 74.1%. Appearance was a moderately sensitive parameter (Table 2).

Posture was specific with little expression in groups C_1 (7.4%) and C_2 (3.7%). It was also highly sensitive. Three pain levels were identified in the *E-groups* (E_0, 70.4%; E_1, 51.85%; E_2, 7.3%).

Sweating had good specificity with little expression in groups C_0 (3.4%), C_1 (3.4%) and C_2 (14.8%). In particular, group C_2 responded more strongly, indicating that the epidural analgesic mixture could have a stimulating influence. Sweating was not a sensitive parameter: weak prevalence of 11.1% in group E_1, 7.3% in E_2, and 0% in E_0 (no analysis done).

Head movement showed bad specificity in groups C_1 (11.1%) and C_2 (40.2%) with a possible effect of the epidural analgesic mixture from T_3 to T_{12}. It was very sensitive as well (Group E_0, 59.3%; E_1, 29.6%; E_2, 3.7%).

Kicking at the abdomen was highly specific and was only lightly expressed in group C_2 (3.7%). Expression percentages in the *E-groups* (E_0, 18.5%; E_1, 3.7%; and E_2, 0%) were too low for analysis.

Appetite presented good specificity with little expression in groups C_1 (7.4%) and C_2 (14.8%) but weak sensitivity, allowing the differentiation of group E_0 from group E_2 only.

Pawing on the floor showed good-to-moderate specificity with values other than 0 only present in group C_2 (18.8%), suggesting a slight effect of the epidural analgesic mixture. Pawing was very sensitive since it could differentiate all *E-groups* (E_0, 77.8%; E_1, 33.3%; E_2, 3.7%).

3.3.3. Responses to treatment

Interactive behaviour was found specific, with a slight modification from normal behaviour only in group C_1 (9%). It was not a sensitive parameter: weak prevalence of 24% in group E_0 alone (no analysis done).

The *response to palpation of the painful area* appeared to be very specific with no expression (no values other than 0) in the *C-groups* and expression >37% in the *E-groups*. It was also very sensitive, allowing all *E-groups* to be distinguished from each other.

3.4. Complementary physiological criteria

NIBP did not change with time for the *C-groups* ($P > 0.2$). In *E-groups*, only E_0 values varied significantly with time ($P = 0.01$). Values in this group were significantly higher than in group E_1 at $T_{2,3,8}$, and group E_2 at $T_{2,3,5-8}$. In group E_2, mean NIBP was significantly lower than in group E_1 at T_{4-6}, and there was no difference between group E_2 and the *C-groups* ($P > 0.27$). While there was no variation with time for all groups except E_0, average values (in mmHg) of mean NIBP were for each group: C_0, 79 ± 9.1; C_1, 86.5 ± 9.4; C_2, 79.7 ± 10.4; E_0, 118.1 ± 11.6; E_1, 97.2 ± 10.5; E_2, 84.7 ± 8.8. This parameter allowed to statistically differentiate *E-groups* between each other at many time-points. Finally, there was a high positive and significant association ($P < 0.0001$) between total CPS and mean NIBP: for each increment of 1 unit in NIBP, CPS increased by 0.18 units. Mean NIBP was a very specific and sensitive parameter of orthopaedic pain.

Mean *glucose* values were significantly higher in group C_2 than in group C_0 at $T_{0,2,4}$ and group C_1 at T_0. There was no significant difference with time and between *E-groups*, suggesting the marker was neither specific nor sensitive. It also suggested an effect of the epidural analgesic mixture on blood glucose.

Cortisol did not vary as a function of time or group in *C-groups* ($P > 0.46$), but showed variation in the *E-groups*. Significant change occurred with time in group E_0, and values reached a maximum at T_8, which was also the case for the total CPS in this group. There was a positive and significant association ($P < 0.002$) between total CPS and cortisol: for each increment of 1 unit in blood cortisol, the CPS increased by 0.095 units. Moreover, cortisol in group E_2 was significantly lower than in group E_0 at T_8 ($P < 0.0001$) and group E_1 at $T_{0,4}$ and $_8$ ($P < 0.007$). While there was no variation with time for all groups except group E_0, average values (in nmol/L) of cortisol were for each group: C_0, 82 ± 21.1; C_1, 112.7 ± 30.4; C_2, 101.7 ± 27.8; E_0, 106 ± 16.8; E_1, 140.6 ± 33.5; E_2, 69.7 ± 18.8. Cortisol was specific and moderately sensitive to orthopaedic pain in this experimental model.

3.5. Analgesia efficacy (Figs. 3 and 4)

The average number of epidural injections (saline for group E_0, analgesic mixture for groups E_1 and E_2) varied greatly ($P = 0.048$) between groups: E_0, 10.7 ± 2.3; E_1, 3 ± 2.6 with one horse requiring six injections; E_2, 1 ± 1. All horses from group E_0 required phenylbutazone 6.3 ± 2.1 h after T_0, but none in groups E_1 or E_2 did.

In group E_0, a first injection of phenylbutazone (CPS $\geqslant$ 20/39) at $T_{4,7,8}$ allowed to decrease CPS, respectively, at the following level: 14 in 6 h, 16 in 4 h, and 15 within the hour. But, CPS stayed high and did not go below the intermediate level of pain (13/39) before T_{18}, T_{19} and T_{16}, respectively.

In groups E_1 and E_2, where epidural analgesics were used in a pre-emptive fashion, no supplemental NSAID injection was required, and the delay between the pre-emptive epidural injection and its first re-injection was (in hours) 7.7 ± 2.1 in group E_1, 17.3 ± 9.1 in E_2, and 3.6 ± 1.5 in E_0 ($P = 0.044$). Furthermore, in group E_1, CPS scores decreased very effectively with the epidural re-injection in 1 h after one re-injection for one horse, after two re-injections in 2 h for a second horse, whereas it took six epidural injections over the whole 24-h period to keep the third horse at lower level of CPS. Finally, in group E_2, one horse required epidural re-injection at T_7 and T_{14}, another received one epidural re-injection at T_{21}, and one did not require any rescue analgesia.

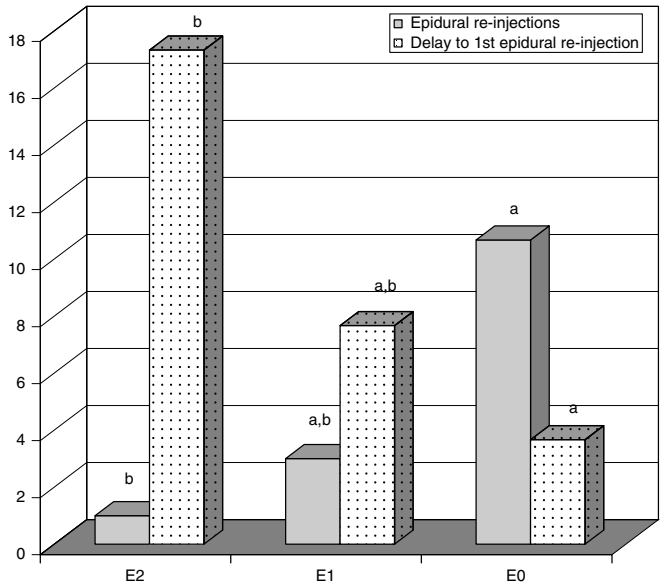

Fig. 4. Average number and delay for epidural re-injections in each *E-group*. The average number of epidural re-injections (either saline in group E_0, or analgesics mixture in groups E_1 and E_2) is represented in association with the average delay for such injection required in each *E-group* when CPS $\geqslant$ 13/39. Different letters indicate significant differences between groups for each parameter.

4. Discussion

The first studies evaluated metabolic and endocrine repercussions of general anaesthesia and surgery in horses (Robertson, 1987; Robertson et al., 1990; McCarthy et al., 1993). Raekallio et al. (1997) used a CPS to evaluate post-arthroscopic pain in a placebo ($n = 10$) and a pre-emptive phenylbutazone ($n = 15$) group. The authors highlighted the poor correlation between subjective and objective behavioural measurement of pain. In a subsequent identical arthroscopy study evaluating pre-emptive epidural of morphine–detomidine ($n = 4$) and placebo ($n = 4$) efficacy, Goodrich et al. (2002) found acceptable within – but poor between –observers agreement, using subjective numerical rating on video recording. Pritchett et al. (2003) used a subjective numerical rating scale to evaluate physiological and behavioural indicators of pain in a control group ($n = 10$), a non-painful anaesthesia group ($n = 10$) as well as a surgical correction of gastro-intestinal disorder group using pre-emptive flunixine administration ($n = 7$). From these studies, it has been recognized that, in absence of any validated scale, the efficacy of subjective equine pain scales is heavily influenced by the ability of the observer to recognize pain. Particularly, it could be very difficult to extend their use to other practitioners with regards to the personal experience in pain evaluation influencing the development and use of each scale.

Finally, using direct observation at set time-points and time lapse video recording to produce activity budgets, Price et al. (2003) determined some abnormal behaviours between surgery (arthroscopy under multimodal analgesia and general anaesthesia, $n = 6$) and control (non-painful general anaesthesia, $n = 6$) groups. However, as for the study of Pritchett et al. (2003), the effects of any surgery and general anaesthesia on behaviour were still unclear and could not be accounted for, thereby reducing the reliability of these findings as specific indicators of post-operative limb or abdominal pain. Rietmann et al. (2004) also performed automated behavioural video-analysis, but their study was based on stress response quantified by comparing horses' heart rate variability between rest and exercise activity.

The current study combines various methods of pain evaluation to construct a CPS as reliable and precise as possible. Constant and repeated evaluations by multiple observers of the behavioural parameters allowed us to reproduce multiple evaluations of the same parameter. Results were identical, thereby confirming the reproducibility of each parameter. To add an objective dimension to the evaluation, video analysis permitted infallible data recording. It also allowed repeated visualization of sequences, giving more precision to the evaluation of the parameters studied. This video analysis permitted non-direct evaluation of horses without the presence or intervention of humans. We believe that this element could allow future discovery of animal behaviours different from those expressed in the presence of humans.

The current study first revealed that behavioural parameters of the CPS were all repeatable among observers in a good-to-excellent way. Groups C_0 and C_1 were selected for intra-observer evaluation as they were considered exempt from any drug effect so that observer consistency could be properly assessed.

From the viewpoint of specificity, further analysis including a series of 3 negative control groups, has allowed to determine (i) the limited effect of sedation with acepromazine and romifidine on the CPS; (ii) the local and systemic actions of an epidural mixture composed of ropivacaine, detomidine, ketamine and morphine as well as their effects on the parameters; and (iii) the specificity of the 13 parameters of the original CPS proposed in this study.

All groups were homogenous in their degree of response to sedation, and we could reasonably consider that it did not influence the results. Statistical analysis of the *C-groups* allowed suspecting some specific effects of the epidural analgesic mixture: slight increase in respiratory rate, clear depression of the gastrointestinal transit/motility, and stimulation of pawing on the floor (possible ropivacaine effect), of sweating, glucose, and head lowering. For the three latter effects, we suspect a major role of detomidine with regards to such recognized systemic actions (Lerche et al., 1993) correlated with the pharmacokinetic data available for this drug after its epidural administration (Sysel et al., 1996).

Response to palpation of the painful area and *kicking at abdomen* were criteria with excellent specificity, followed by *interactive behaviour* and *posture* as excellent-to-good. Two of these four parameters were also classified with an excellent sensitivity. At the opposite, criteria such as *rectal temperature* and *appearance* had null specificity, being expressed in all *C-groups*. For rectal temperature we could suspect that the placement of an epidural catheter would interfere with the tonus of the anal sphincter (and also with the administration of the local anaesthetic ropivacaine specifically in the group C_2). Poor specificity of *appearance* can only be explained by a wrong description of the parameter in the first place. To increase its specificity and sensitivity, its description would have to be modified and it would necessitate a re-validation in another similar study. With an intermediate crescendo classification in specificity, we will find criteria such as *head movement*, *digestive sounds*, *sweating*, *pawing on the floor* and *appetite*, which present the particularity of a quasi-exclusive expression in the group C_2, being only affected by epidural drugs action.

Statistical analysis of the results obtained with the CPS indicates that its global sensitivity was good. This is important, since it represents a validation of the experimental approach and model in designing three different pain intensities. The most sensitive parameters were *response to palpation of the painful area*, *pawing on the floor*, *head movement* and *posture*. All these parameters were also cited in the review by Ashley et al. (2005) who clearly demonstrated their interest in behavioural orthopaedic pain quan-

tification in horses. Indeed, from various equine painful syndromes, there is widespread agreement on behavioural indicators for recognition purposes, and complex scoring systems exist to describe and analyze lameness (Ashley et al., 2005). The most commonly cited behavioural signs of acute limb/foot pain are altered weight distribution and altered limb loading/position (Stashak, 2002; Price et al., 2003). These were included in the parameters *posture* and *pawing on the floor*. A significant increase in lowered head carriage was also observed in post-arthroscopic studies (Price et al., 2003). Animals experiencing pain have increased sensitivity to aversive stimuli and, consequently, a lowered threshold to subsequent stimulation (hypersensitization). Nociceptive withdrawal threshold testing has been and remains a common method of determining changes in the sensitivity of various tissues to noxious and non-noxious stimuli in addition to the evaluation of analgesic drug efficacy. The results obtained with the *response to palpation of the painful area* in this study and others (Wolf, 2002) corroborate the hypothesis that a mechanical device could be developed in the future for precise quantification (Chambers et al., 1993). Therefore, it was not surprising to find these parameters as most sensitive.

Heart and *respiratory rates*, defined *appearance*, *appetite* and *interactive behaviour* had moderate sensitivity, as they were able to differentiate two levels of pain. *Appearance*, as defined originally, was found unsatisfactory for specificity and moderate for sensitivity. With regards to its high occurrence in the literature as a potentially useful parameter, we suspect its description was non-valuable in the first place. *Interactive behaviour* was also disappointing, particularly in relation to promising initial results (Price et al., 2003). *Appetite* was definitively modified in the presence of pain, but it might be difficult to graduate its variation. On the other hand, it is usually simple to identify when the animal does not eat at all. Observation of animal behaviour to obtain a better description of this parameter might help to increase its sensitivity. No significant difference in equine heart rate has been found between pain and control groups in wound sensitivity (Redua et al., 2002) or peri-operative analgesia studies (Raekallio et al., 1997; Dzikiti et al., 2003; Price et al., 2003). As other studies in horse (Price et al., 2003) and dog (Holton et al., 1998), we observed that *heart* and *respiratory rate* changes could not predict the pain level. This is important as these physiological factors were reported by equine practitioners as main indicators of the presence of clinical pain (Price et al., 2002).

For the first time, to our knowledge, *NIBP* was evaluated and was shown to be an excellent parameter to assess equine orthopaedic pain because of its good specificity and high sensitivity. We believe it would add great value to a pain scale.

Of particular interest is the statistical correlation observed between the CPS and both *NIBP* and *cortisol*. Pritchett et al. (2003) found *heart rate* and *cortisol* to be significantly higher in post-operative exploratory celiotomy cases compared to controls throughout a 30-h period. These parameters were also associated (as in our study) with significant differences in pain scores between case and control groups, although no correlation was established. Intrinsic changes (diurnal variations) are very important in the horse (Hillyer et al., 1992; Dybdal et al., 1994; Sojka and Levy, 1995; Beech, 1999; Levy et al., 1999), but *cortisol* can help to identify painful situations if frequent measurements are taken during follow-up. More frequent measurements might be needed to make it a useful parameter to validate a pain scale or to assess pain experimentally, but the delay between sampling and results makes this parameter unacceptable for practical pain scale use. The clinical relevance of these two criteria need confirmation with regards to the present results got on an experimental orthopaedic pain model. Their specificity and sensitivity to various levels of clinical pain need to be validated. Their main limitations are availability and practicability in a field position.

Glucose was the least sensitive of all markers, and its specificity was poor. In all groups, an increase in glucose was reported after i.v. administration of romifidine. In the horse, this is thought to be the result of α-2 adrenoreceptor-mediated depression of insulin release from β-cells (Greene et al., 1987). Cortisol may play a permissive role in the hyperglycaemic response in humans (Traynor and Hall, 1981; Lacoumenta et al., 1987). The absence of hyperglycaemia in the present study may be a result of the limited plasma cortisol elevation. *Glucose* is definitively not a good parameter to precisely assess pain in horses.

In this study, key specific and sensitive behavioural indices relating to the *response to palpation of the painful area*, *posture*, and to a lesser value *pawing on the floor* were identified as potentially most useful for inclusion in a CPS for the assessment of equine acute orthopaedic pain. *Kicking at abdomen* is a potential candidate to be included in such CPS with confirming data on its sensitivity, whereas *interactive behaviour* was deficient in sensitivity, and *head movement* in specificity to be included in such CPS. The parameter *appearance* would need to be re-evaluated after modification of its description. It is established that physiological criteria are of low interest for pain evaluation in horses, except for *NIBP*.

Following our second objective, this study stated without any doubt that the most efficient analgesic protocol was multimodal and pre-emptive (Group E_2), indeed providing a statistical absence of difference ($P > 0.09$) for total CPS between groups E_2 and C_2. This suggests that the protocol used in Group E_2 gave almost complete analgesia. Its degree of response was about three times more powerful (requiring three times less rescue analgesia) and of longer duration than pre-emptive monotherapy (Group E_1). Finally, in the current study, the post-injury approach, based on a unique NSAID, corroborated its efficacy found in clinical evidence of inflammatory pain syndromes. However, this efficacy takes time to establish and cannot be

compared with the other protocols tested in this study. Research in humans has established the superior efficacy of multimodal and pre-emptive analgesia; it is now appreciable to have reproduced such clear evidence in horses. First, pain therapy must be tailored to the individual animal. Second, the therapeutic modality must be selected based upon suspected mechanisms and an assessment of the severity and type of pain (superficial, deep or visceral) being treated. Severe pain originating from a peripheral site, and development of central sensitization as suggested by secondary hyperalgesia, require the administration of more potent analgesics or combinations of analgesic drugs (multimodal therapy) acting by different mechanisms of action (Muir, 2005). Administration of a single analgesic drug should not be expected to provide adequate pain relief in all instances. Third, pain treatment and analgesic drugs administration should be anticipated in all surgical and emergency patients. This implies that analgesic drugs should be administered before the pain-producing event (pre-emptively) whenever possible. Pre-emptive analgesic therapy helps to suppress stress-related consequences of acute pain and decreases the likelihood of developing central sensitization, which can contribute to the development of chronic pain states. Fourth, analgesic therapy should be continued for as long as required; administration of a single dose of an analgesic drug is unlikely to produce adequate long-term analgesic effects and does not provide adequate effects on the sustained pain associated with post-traumatic or surgical events. Analgesic drugs should be administered for a minimum of three days after routine elective surgical procedures (Muir, 2005).

5. Conclusion

Despite the small number of study subjects, we have been able to describe the specificity and sensitivity of many parameters and to identify three levels of pain. Such an approach could potentially be reproduced with other pain models and in other species. This study confirmed that physiological parameters are not valid to evaluate orthopaedic pain in horses, even if *heart rate* could be indicative in some circumstances (e.g., moderate to intense acute pain). A promising finding of this study is the high potential as specific and sensitive parameter of *NIBP*. Of all behavioural parameters to be included in a CPS for orthopaedic pain in horses, *posture* is the first one, followed by *pawing on the floor*, and possibly head movement, kicking abdomen and appearance. Inclusion of a response to care category, as already done for small animals and other species, reveals to be an interesting pain evaluation tool for horses (Price et al., 2003). In this category, *response to palpation of the painful area* was found to be very specific and sensitive in our study. The validation of all these behavioural parameters has led to the establishment of a strong, reliable CPS. As a simple tool to evaluate orthopaedic pain in horses, it should facilitate pain assessment in this species. The next step would be a comparison to objective three-dimensional kinematic gait analysis and/or ground reaction forces (force plate) analysis in clinical setting.

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