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Original Article

The lipopolysaccharide model for the experimental induction of transient lameness and synovitis in Standardbred horses

E. Van de Water ^{a,*}: Eline.VandeWater@UGent.be¹, M. Oosterlinck ^a, N.M. Korthagen ^{b,c}, L. Duchateau ^d, M. Dumoulin ^a, P.R. van Weeren ^b, J. Olijve ^e, D.A. van Doorn ^{b,f,g}, F. Pille ^a

^a Department of Surgery and Anaesthesiology, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium

^b Department of Equine Sciences, Faculty of Veterinary Medicine, Utrecht University, Yalelaan 112, 3584 CM Utrecht, The Netherlands

^c Department of Orthopaedics, University Medical Center Utrecht, Heidelberglaan 100, 3584 CX Utrecht, The Netherlands

^d Biometrics Research Group, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium

^e Rousselot, Meulestedekaai 81, 9000 Gent, Belgium

^f Department of Farm Animal Health, Faculty of Veterinary Medicine, Utrecht University, Yalelaan 7, 3584 CL Utrecht, The Netherlands

^g Equivado, Equine Nutrition Consultancy, Marnixlaan 80, 3552 HG Utrecht, The Netherlands

¹ Current address: DAP Equinox BV, Vekemans 10, 2460 Kasterlee, Belgium

*Corresponding author. Tel.: +32 9 264 76 18.

Highlights

- Lipopolysaccharide (LPS; 10 endotoxin units) in the middle carpal joint induced lameness
- Peak lameness was observed at 4 h post-injection, returning to baseline within 48 h
- Lameness was prevented by meloxicam daily for 4 days prior to LPS injection
- Pre-treatment with meloxicam reduced prostaglandin(PG)E₂ in synovial fluid
- LPS for injection requires uniform preparation and handling

Abstract

An established lipopolysaccharide (LPS) model previously described in Warmbloods, was inconsistent in Standardbred horses, where lameness was not detected despite the presence of synovitis. The present study aimed to determine the dose of LPS from *E. coli* O55:B5 required to induce mild to moderate lameness following middle carpal joint injection in Standardbred horses and to quantitate the induced lameness over time, with and without anti-inflammatory pre-treatment. In a baseline trial, eight healthy, clinically sound Standardbred horses were used in a rule-based dose-escalation design trial, starting at a dose of 10 endotoxin

units (EU). Lameness at trot was evaluated visually and quantitatively (using an inertial-sensor system and pressure plate analysis). Synovial fluid aspirates were analysed for total nucleated cell counts, total protein and prostaglandin E₂ (PGE₂). Following 2 months wash-out, the effective LPS-dose determined in the baseline trial was used to evaluate the effect of anti-inflammatory treatment. A mixed model for repeated measures with horse as random effect was used for analysis.

After injection of 10 EU LPS, the desired degree of lameness was observed in the baseline trial, with maximal lameness at post-injection hour (PIH) 4, followed by a rapid decline and return to baseline by PIH 48. No lameness was observed following pre-treatment with meloxicam. In synovial fluid, PGE₂ was significantly higher at PIH 8 and PIH 24 in the baseline trial compared with following meloxicam pre-treatment. In conclusion, injection of the middle carpal joint with 10 EU LPS consistently induces a transient lameness and synovitis in Standardbred horses.

Keywords: Arthritis; Joint disease; Lameness; Lipopolysaccharide; Synovitis

Introduction

Responses to an established lipopolysaccharide (LPS) model previously described in Warmbloods have been inconsistent in Standardbred horses. The model was previously documented by de Grauw et al. (2009a,b). In those studies, 0.5 ng endotoxin (LPS) of *E. coli* O55:B5 (L5418, Sigma-Aldrich) was injected intra-articularly, inducing a mean lameness score of three, on a scale from zero (sound) to five (severe lameness), at 8 h post-injection in Warmblood horses. However, when using the same amount and type of LPS was used to induce acute synovitis of the right middle carpal joint in 24 Standardbred horses, the expected degree

of lameness was not observed, despite the obvious presence of inflammation (Van de Water et al., 2017).

A literature review revealed a wide variability in the reported timing of lameness evaluation moments post LPS infiltration. In most studies in which the LPS model has been used, observations have not been performed earlier than post-injection hour (PIH) 6-8 (Palmer and Bertone, 1994; Gottschalck et al., 1998; Khumsap et al., 2003; Ishihara et al., 2005; Kay et al., 2008; de Grauw et al., 2009a, b; Pearson et al., 2011; Van Loon et al., 2013; de Grauw et al., 2014; Williams et al., 2016; Cokelaere et al., 2018) or the specific timing of peak lameness post injection has not been reported (Jacobsen et al., 2006; Guedes et al., 2018). In studies in which lameness evaluation was performed at earlier time points, the lameness peak has generally been observed at PIH 4 (Lindegaard et al., 2010; Van Loon et al., 2010; Ross et al., 2012; Van Loon et al., 2012; Andreassen et al., 2017). In the study by Lindegaard et al. (2010) horses were treated with morphine at PIH 4, potentially masking a further increase in lameness. In one of the studies evaluating lameness earlier than PIH 6-8, peak lameness was only observed at PIH 12 to 24 (Lucia et al., 2013). Peak lameness has also been reported to extend from PIH 3 to 8 (Carregaro et al., 2014).

Furthermore, large variations in dosages for the induction of synovitis and lameness with LPS have been used, ranging from 0.125 ng to 5000 ng of the same serotype of *E. coli* (O55:B5; Palmer and Bertone, 1994; Khumsap et al., 2003; Ishihara et al., 2005; Jacobsen et al., 2006; Kay et al., 2008; de Grauw et al., 2009a, b; Meulyzer et al., 2009; Santos et al., 2009; Lindegaard et al., 2010; Van Loon et al., 2010; Pearson et al., 2011; Ross et al., 2012; Van Loon et al., 2012; Lucia et al., 2013; de Grauw et al., 2014; Andreassen et al., 2017). These

discrepancies in the literature raise questions about both the reported dosages and the evaluation time points of this experimental model, warranting further investigation.

The first question is whether previously reported concentrations are accurate. Usually manufacturers of LPS solutions do not specify the exact concentration of the stock solution and only provide a qualitative indication of the concentration. In addition, the biological activity of LPS can vary widely within the same mass of endotoxin (Associates of Cape Cod Inc., 1997). Therefore, quite different LPS doses might be required to elicit the same biological effects. The use of ‘endotoxin units’ (EU) focuses on the specific activity of the endotoxin and avoids the issue of different potencies. For that reason, the use of EU has been recommended over the use of mass concentrations (Associates of Cape Cod, 1997).

Standardisation of the laboratory procedures for the preparation of the LPS solution are also important. Micelle formation is an important feature of endotoxin molecules (Bergstrand et al., 2006), potentially affecting its activity and requiring consideration during preparation. It is also known that varying LPS extraction methods result in different LPS products and the exact LPS product of *E. coli* O55:B5 used for this type of experimental study has not been consistently specified. Finally, the equine breed(s) and/or joint investigated may also differ substantially in their biological responses.

The aims of this study were: (1) to determine the dose of LPS in EU (derived from *E. coli* O55:B5 using standardised laboratory preparation) required to induce mild to moderate lameness following middle carpal joint injection in Standardbred horses that is inhibited by pre-treatment with anti-inflammatory; and (2) to quantitate the induced lameness over time, with and without anti-inflammatory pre-treatment.

Materials and methods

The study was approved by the ethical committee of the Faculty of Veterinary Medicine of Ghent University (Approval number, 2015/52; Approval date, 11 June 2015).

Study design and timing

The first part of the study ('baseline trial') was based on a rule-based dose-escalation study (Le Tourneau et al., 2009). A post-hoc analysis of LPS enzymatic activity in the stock solution used in the previous study (Van de Water et al., 2017), revealed that the middle carpal joints had been injected with approximately 5 EU LPS. Therefore, the present study started with a dose of 10 EU. In cohorts of two horses, this starting dose was injected into the right middle carpal joint and was increased or decreased in subsequent cohorts if lameness was either too mild to be detectable or too severe ($>4/5$), respectively. When the desired degree of lameness was obtained, additional cohorts of two horses were injected and evaluated with the same dose of LPS, until a total sample size of eight horses was reached. The sample size was calculated based on results of a previous study (Van de Water et al., 2017) with a statistical significance of 0.05 and power of 0.8.

Lameness was evaluated subjectively by two experienced clinicians, and objectively by an inertial sensor system, every 2 h during the first 12 PIH, every 4 h during the next 12 PIH, and every 12 h during the last 24 PIH. Based on the results of de Grauw et al. (2009b), which served as a basis for the present study, the horses were also subjected to pressure plate analysis at PIH 0, 8, and 24.

Immediately prior to LPS-injection (PIH 0) and at PIH 8, 24 and 48, synovial fluid was sampled and analysed for total protein, total nucleated cell count and prostaglandin E₂ (PGE₂)

concentration (de Grauw et al., 2009 a, b). At the same time points, jugular venous blood samples were collected for hematology. In addition, the horses were under constant veterinary surveillance, with assessment of clinical parameters including respiratory rate, heart rate, rectal temperature, appetite, fecal output, signs of colic and other discomfort, although this was not quantified using a published pain scale.

Following a wash-out period of 2 months, the determined LPS-dose of the baseline trial was injected into the same eight horses, which had now received 4 days of non-steroidal anti-inflammatory treatment with meloxicam (0.6 mg/kg PO SID; Metacam, Boehringer Ingelheim) prior to LPS-injection ('meloxicam trial'), as described previously by De Grauw et al. (2009a). This medication was continued during the test period. Subjective and objective lameness assessments, along with synovial fluid and jugular venous blood sampling were performed as described above.

Since prior work has demonstrated the ability of meloxicam to significantly downregulate the inflammatory response to LPS (de Grauw et al., 2009a,b), this non-steroidal anti-inflammatory (NSAID) was used to test the usability of the revised LPS model for the future evaluation of substances with a claimed preventive anti-inflammatory effect. Due to the short-acting inflammatory effect of the injected LPS, the authors believe that this model is especially useful when evaluating the preventive rather than curative effects of a wide range of anti-inflammatory treatments, such as nutritional supplements, even though the model has already been used successfully in numerous curative treatment trials (Santos et al., 2009; Van Loon et al., 2010; Van Loon et al., 2013; Carregaro et al., 2014). Therefore, pre-treatment with meloxicam was preferred over curative treatment in the present study.

Throughout the study, humane endpoints were based on lameness and systemic signs of inflammation. In cases of severe lameness ($>4/5$ on a scale from zero [sound] to five [non-weightbearing lameness]), or in cases where any severe systemic clinical signs were observed, such as fever, tachycardia, tachypnea, abdominal discomfort, diarrhea or anorexia, withdrawal from the experiment and provision of rescue medication (NSAID) and further appropriate veterinary care would have been provided.

Horses

Eight healthy and clinically sound French Standardbred horses were used (mean $\pm$ SD age 4 ± 0.5 years; body mass 470 ± 24 kg). Horses with a known history of musculoskeletal problems were excluded. All horses underwent routine farriery and were given time to adjust to the research environment, one week prior to each trial date. During the study period, all horses were individually housed under identical circumstances and received a standard ration of hay and concentrate feed.

Lameness evaluation

Lameness was evaluated subjectively (routine visual examination) and objectively (inertial-sensor system and pressure plate) at trot, at the time-points indicated above. Routine visual examination was performed at trot on a straight line on a hard surface, by two experienced clinicians, giving a consensus score from 0 (sound) to 5 (non-weightbearing lameness).

Pressure plate (PP) measurements were performed using a dynamically calibrated pressure plate (Footscan 3D 2m, RSscan International) mounted on top of a force plate (BP4602070RS-2K, AMTI). Analysis was performed as described by Oosterlinck et al. (2012).

The following variables were calculated: (1) peak vertical force (PVF, N/kg); (2) vertical impulse (VI, Ns/kg); (3) stance time (ST, ms). For each set of five measurements, all left fore (LF) and right fore (RF) measurements were averaged, and subsequently, PVF, VI and ST ratios between both forelimbs were calculated as -

$$\%RF = RF/(LF+RF) \times 100\%$$

Using this approach, a value of 50 indicated a perfect symmetry between left and right, whereas a value higher or lower than 50 indicated relatively higher loading of the right or left limb, respectively.

Inertial sensor system (ISS) measurements were performed using the Equinosis Q system (Equinosis)¹ at trot in a straight line on a hard surface, with a sensor attached on the head, the pelvis and the right forelimb, as specified by the manufacturer. At least 25 strides were analysed with dedicated software (Lameness Locator 2014 v.2, Equinosis²). Mean forelimb asymmetry was expressed as the vector sum (VS, mm) of the difference in minimal and maximal head height between the right and left forelimb during a stride, with a threshold of 8.5 mm for distinguishing between sound and lame horses (Keegan et al., 2011). In the present study, a positive value was used for RF lameness, and a negative value for LF lameness.

Preparation of LPS

The initial stock solution of LPS from *E. coli* O55:B5 (L5418, Sigma Aldrich; lot 045M4029V) had a concentration of 3,000 EU/mL, as quantified using a recombinant factor C assay (EndoZyme II assay, Hyglos). For every cohort of two horses, an LPS-solution containing an appropriate dose of LPS (starting with 10 EU/mL for the first cohort) was prepared, no greater than 1 h prior to injection to ensure stability of the dilution. Before dilution, the stock

¹ See: Equinosis Q system. <https://equinosis.com/veterinarians/learn-more/> (Accessed 25 January, 2021).

² See: The Equinosis Q with Lameness Locator. <https://equinosis.com> (Accessed 25 January, 2021).

solution was vortexed for 10 min at a speed of 1500 rpm to break down endotoxin micelles and achieve a homogenous solution. Subsequently, the solution was diluted in sterile isotonic saline with micropipettes under laminar flow and on ice. Between every consecutive dilution step, the solution was vortexed for 2 min at 1500 rpm. All LPS solutions were stored in glass vials at 4 °C. Immediately before injection, the final solution was vortexed again for 2 min.

Sampling and sample analysis

Following sedation with detomidine (Detogesic, Vetcare) and butorphanol (Torbugesic, Zoetis), both at a maximal dose of 10 µg/kg IV, synovial fluid samples (3.5 mL) were collected by aseptic arthrocentesis of the right middle carpal joint using a 21G 4 cm needle. Samples were immediately divided into two sterile containers; 1 mL was collected into an EDTA-coated tube and the remaining fluid was collected in a plain tube. Samples were immediately stored at 4 °C and analysed (EDTA) or processed (plain tube) within 1 h following collection. The EDTA-sample was analysed for total nucleated cell count (TNCC, $\times 10^3$ cells/µL) with a hematology analyser (Scil Vet abc Plus⁺, Scil Animal Care Company; Van de Water et al., 2016) and total protein (TP, g/L) by spectrometry. The plain tubes were centrifuged at 600 *g* for 20 min and the supernatant was divided in aliquots of 500 µL, which were immediately stored at -80 °C within 2 h following sample collection, for subsequent PGE₂-analysis. PGE₂ was determined by HPLC-MS/MS analysis on an HPLC system (PerkinElmer LC200, Perkin Elmer) coupled to an electrospray ionisation linear ion trap quadrupole mass spectrometer (4000 Q TRAP, Applied Biosystems). The instrument was operated in negative MRM mode. For extraction, 0.1 M sodium acetate buffer (pH 5) and ethyl acetate were added to 100 µL of sample. The organic phase was then separated during two consecutive freeze cycles. After vacuum centrifuging, the residue was dissolved in methanol and measured. All samples were

normalised to an internal standard (16,16-dimethyl PGF₂ α) and PGE₂ concentrations (pg/mL) were calculated from a standard curve of known concentrations.

Within the context of safety assessment of LPS, blood samples were taken from the left jugular vein with a 21G, 4 cm needle in EDTA-coated vacutainers. Samples were immediately refrigerated at 4 °C, and within 1 h after collection subjected to hematologic analysis with a hematology analyser (scil Vet abc Plus⁺, Scil Animal Care Company).

Statistical analysis

Statistical analysis was performed with SAS version 9.4. Global significance was set at 5%. A mixed model with horse as random effect, and time, treatment and their interaction as categorical fixed effects was performed. The treatment effect was also tested at different time points separately for TNCC and TP at PIH 0, 8, 24 and 48, for %RF(PVF) and %RF(ST) at PIH 0, 8 and 24 and for VS at PIH 0, 2, 4, 6 and 8, using the Bonferroni correction for multiple comparisons. As PGE₂ and %RF(VI) data presented significant departures from a normal distribution, that could not be resolved after logarithmic transformation, a Wilcoxon rank sum test was used and the treatment was tested at the different time points using the Bonferroni correction for multiple comparisons.

Results

Lameness evaluation

In all cohorts, the initial dose of 10 EU induced lameness within the predetermined range, obviating the need for further dose adjustments. Median clinical lameness scores and mean inertial sensor system data for PIH 0, 2, 4, 6 and 8 are presented in Table 1 and 2 respectively. Apart from one horse, the baseline trial lameness peaked at PIH 4 with a median clinical lameness score of 3 (interquartile range 3-3) and a mean vector sum of 38.7 ± 20 mm.

Six horses presented a maximal lameness grade of 2-3/5 (mean $\pm$ SD VS 36.8 ± 8.5 mm), with one horse showing a peak lameness of grade 4/5 (VS 78.8 mm). In the final horse, lameness had already peaked at PIH 2 and only reached grade 1/5 (VS 15.6 mm). In all horses, lameness declined rapidly after the peak and returned to baseline levels by PIH 48 (Fig. 1). In the meloxicam trial, LPS injection could not provoke any relevant or significant change in locomotor symmetry.

Pressure plate data are presented in Table 3. At PIH 0, no significant differences were observed between both trials. VS was significantly higher in the baseline trial at PIH 2 and 4 ($P=0.004$ and $P<0.0001$, respectively), compared to the meloxicam trial. For pressure plate variables (%RF of PVF, VI and ST), no significant changes were observed following LPS injection, and no significant differences between either trial could be observed at any time point.

Synovial fluid analysis

All synovial fluid data are presented in Table 4. At PIH 0, no significant differences could be observed between the baseline trial and the meloxicam trial for any synovial fluid parameter. After LPS injection, there was a significant increase in TNCC and TP in both trials ($P<0.0001$; Fig. 2a, b), whereas PGE₂ increased significantly in the baseline trial but not in the meloxicam trial ($P<0.0001$; Fig. 3). TNCC and TP did not differ significantly between either trial at any time point. However PGE₂ was significantly higher at PIH 8 ($P=0.021$) and 24 ($P=0.030$) in the baseline trial compared with the meloxicam trial.

Possible systemic effects of LPS injection

Respiratory rate, heart rate, rectal temperature and hematology parameters remained within normal limits at every time point.

Discussion

The present study revealed that injection of 10 EU of LPS of *E. coli* O55:B5 in the middle carpal joint of Standardbred horses consistently induces synovitis and lameness. The lameness grade was in agreement with our predefined criteria. As it generally concerned a moderate and transient lameness and synovitis, it seemed acceptable in terms of animal welfare, although the latter certainly comprises more than only lameness grade, and further measures to assess this should be included in the future.

Due to the first LPS-dose being suitable, no further doses were tested and therefore it is unknown whether a lower or higher dose could also have produced a suitable degree of lameness. The induced lameness in the present study was reasonably consistent at the moment of peak lameness, but with one horse being grade 4/5 and one horse being only grade 1/5 lame, it seems reasonable to presume that a higher or lower LPS dose would have resulted in either excessive or too mild lameness, respectively. The use of live animals comprises an inherent degree of biological variability. As all external influencing factors were maximally limited in the present study, the remaining variability may be due to an individual susceptibility to LPS stimulation, thus inherent to the LPS model.

Pre-treatment with meloxicam was consistent in avoiding an increase in visual grade and objective (VS) lameness score following LPS injection. Meloxicam treatment was specifically initiated 4 days prior to LPS-injection based on previous work of De Grauw et al. (2009a), as the authors believe that the model is particularly useful in providing information regarding preventive effects of treatments (for example nutritional supplements with presumed anti-inflammatory properties that can be administered during daily training), due to the very short duration of inflammation induced by LPS injection. This pre-treatment with meloxicam was already well-documented and shown to successfully to inhibit inflammation and lameness

response following LPS injection (De Grauw et al., 2009a), contributing to the statistical validity of the model.

With the dose of 10 EU LPS, a significant difference between the baseline and the meloxicam trial was observed for PGE₂ concentration in synovial fluid after LPS injection, but not for TNCC and TP. These findings are consistent with the results of de Grauw et al. (2009a,b) reporting a sharp reduction in PGE₂ concentration when LPS is injected following oral meloxicam treatment compared to LPS alone, but no significant effect on white blood cell count, total protein, or percentage of neutrophils. The question arises as to what extent this increase in synovial fluid parameters is due to repeated arthrocentesis and/or injection of a substance into the joint. However, repeated arthrocentesis reportedly only has a very mild impact on synovial fluid cytology, of no clinical importance (Sanchez Teran et al., 2012; Rinnovati et al., 2017). Even though it could affect synovial biomarker levels confounding assessment of joint disease in clinical cases (Van den Boom et al., 2004, 2005), in the LPS model this effect is overshadowed by the effect of LPS (Ross et al., 2012; Lucia et al., 2013). Injection of sterile Ringers lactate solution or sterile saline can also induce mild inflammatory responses that are irrelevant in comparison with the massive response induced by LPS (Gottschalk et al., 1998; Campebell et al., 2004; Ross et al., 2012; Lucia et al., 2013). Therefore, sham-injected joints were not included in the present study.

The observed lameness peak in the present study at PIH 4 is in agreement with studies in which lameness evaluation was also performed at earlier time points than PIH 8 (Van Loon et al., 2010; Ross et al., 2012; Van Loon et al., 2012; Andreassen et al., 2017). To the authors' knowledge, the present study is the first to evaluate the course of lameness after LPS injection in detail using a combination of visual and objective, quantitative analysis. The use of the

inertial sensor system allowed very frequent objective lameness measurements (with 2 h intervals only), which is unique in evaluating the LPS model. In contrast to the inertial sensor system, the pressure plate analysis could not reveal a significant increase in locomotion asymmetry following LPS injection. During the design of the present study, pressure plate evaluation was scheduled at PIH 8 based on de Grauw et al. (2009a,b). The discrepancy in timing between the lameness peak and pressure plate analysis may explain why the inertial sensors were able to detect a lameness (at PIH 2 and 4), in contrast with the pressure plate and force plate combination. The timing can probably also explain the lack of observable lameness in our previous study (Van de Water et al., 2017), in which lameness evaluation was also performed at PIH 8.

The LPS dose determined in the present study was estimated to be about twice the dose used in Van de Water et al. (2017). The present study highlights the importance of the use of endotoxin units (EU) instead of units of mass, in addition to the correct handling and preparation of LPS, to allow for direct comparison between future studies. As the biological activity of LPS can present important variation within the same mass of endotoxin (Associates of Cape Cod Inc., 1997), expressing an LPS-dose in units of mass creates unnecessary variation, which is not the case when using EU. During the handling of LPS, vortexing is very important to break down micelle formation within the solution (Bergstrand et al., 2006) and thus to achieve a homogenous solution for injection. The fact that in many studies, vortexing has not been routinely performed, or at least has not been reported, may be a plausible reason for the large variation between studies. Furthermore, pertaining to the biological effects of LPS, the question arises whether dose and timing are related. It cannot be excluded that the dose of LPS might influence the timing of the lameness peak. For this reason, it is of utmost importance to

evaluate lameness following LPS injection at short intervals from PIH 2 to 12, in order to avoid missing the lameness peak.

Future research is required to evaluate the kinetics of synovial parameters within the LPS model in more detail, including synovial biomarkers other than PGE₂. In this study, the significant differences between the baseline and meloxicam trial for PGE₂ and inertial sensor data, indicate that this revised LPS model is suitable for induction and evaluation of middle carpal joint synovitis and lameness in Standardbred horses. The ability of meloxicam to decrease the concentration of the inflammatory biomarker PGE₂ shows that this model is suitable for testing substances with presumed anti-inflammatory properties. Since the induced synovitis is short lived, the model may be more suitable for assessing the preventive effect of test substances, rather than their curative effect, although it has been extensively used for the latter (Santos et al., 2009; Van Loon et al., 2010; Van Loon et al., 2013; Carregaro et al., 2014).

A limitation of the present study is the use of Standardbred horses, which theoretically may hamper direct comparison with studies using other breeds, for example the Warmblood horses used by de Grauw et al. (2009a,b). However, many different breeds have been used in previous studies, and at least subjectively, no clear associations have been observed between horse breed and reaction to intra-articular LPS injection. A second limitation of this study is that only PGE₂ was used as a biomarker. While PGE₂ is one of the best-known and widely accepted inflammatory biomarkers in synovial fluid, it would be interesting to expand the biomarker panel, as mentioned earlier. A third limitation of this study is the absence of a cross-over design. This was due to the fact that the initial part of the study had to be fully completed to determine the effective dose of LPS, before embarking on the second part (i.e. the comparison with meloxicam). A fourth limitation is the fact that humane endpoints were only focused on

lameness and systemic clinical signs, while measures of animal welfare comprise more than those. The absence of systemic clinical signs and severe lameness alone is not sufficient to state that the model is acceptable in terms of animal welfare, but at least it is a first step. Had severe lameness been induced it would definitely not have been acceptable. However, measures of animal welfare other than lameness should definitely be included in the future. In the past decades, several behavioural pain scales have been developed (Gleerup and Lindegaard, 2016). In addition plasma cortisol and faecal cortisol metabolite measures have been proposed as indicators for animal welfare (Pawluski et al., 2017). A final limitation is that blinded subjective lameness evaluation was not performed. This was due to the fact that decisions regarding increasing or decreasing the LPS dose had to be taken immediately. Therefore, and for the continuity of the study, live assessment was preferred over blinded and randomized video recordings. Moreover, the potential advantage of blinding was considered negligible because the study focused on objective lameness data from an inertial sensor system, of which the statistical analysis was performed blinded.

Conclusion

This study is the first to standardise the use of the intra-articular LPS model for transient synovitis in the horse by using a uniform method of handling LPS solutions (including vortexing) and their specification in EUs. Moreover, this study is the first to report objective lameness assessments at short intervals following LPS injection from PIH 2, demonstrating a consistent lameness pattern after injection of 10 EU LPS of *E. coli* O55:B5 in the middle carpal joint of Standardbred horses, with a mild to moderate lameness peaking at 4 PIH and returning to baseline within 48 h. Horses receiving meloxicam did not show lameness, illustrating that this model is suitable to assess effects of preventive anti-inflammatory treatment. Significant differences in PGE₂ between both trials highlight the discriminatory power and, hence, the

usability of the revised model. This study paves the way for future efficacy studies using this revised model of acute synovitis and lameness to evaluate the preventive effect of different anti-inflammatory treatments.

Conflict of interest statement

D.A. van Doorn was hired as an equine nutrition consultant by Darling Ingredients to coordinate the execution of the project. J. Olijve is employed by Rousselot, a brand of Darling Ingredients. None of the authors has any other financial or personal relationships that could inappropriately influence or bias the content of the paper. The funding company did not participate in analysis or the decision to publish. All authors declare they had full autonomy and independency in research and publishing.

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Table 1 Clinical lameness scores at post-injection hour (PIH) 0, 2, 4, 6 and 8 for both trials (baseline and meloxicam), presented as median (interquartile range).

PIH	Clinical scores	
	Baseline	Meloxicam
0	0.5 (0 – 1)	0 (0 – 1)
2	1.5 (1 – 2)	0.5 (0 – 1)
4	3.0 (3 – 3)	0.0 (0 – 1)
6	2.0 (1.8 – 2.3)	0.5 (0 – 1)
8	2.0 (2 – 3)	0.5 (0 – 1)

Table 2 Vector sum (VS, in mm) data of the inertial sensor system (Equinosis Q with Lameness Locator software ²) measured at post-injection hour (PIH) 0, 2, 4, 6 and 8 for both trials (baseline and meloxicam), presented as mean $\pm$ standard deviation.

PIH	VS (mm)	
	Baseline	Meloxicam
0	6.1 $\pm$ 11.1	9.7 $\pm$ 5.3
2	19.5 $\pm$ 11.2 ^a	2.2 $\pm$ 13.6 ^a
4	38.7 $\pm$ 20.0 ^a	10.4 $\pm$ 10.4 ^a
6	20.6 $\pm$ 11.3	7.3 $\pm$ 11.6
8	23.5 $\pm$ 10.6	10.8 $\pm$ 5.3

^a $\alpha < 0.05$ within time points

Table 3 Pressure plate variables (peak vertical force [PVF], vertical impulse [VI], and stance time [ST]) were measured at post-injection hour (PIH) 0, 8 and 24 for both trials (baseline and meloxicam), and are presented as the mean $\pm$ standard deviation ratio between the left forelimb (LF) and right forelimb (RF), calculated as -

$$\%RF = RF/(LF+RF) \times 100\%.$$

Within time points, no statistically significant differences ($\alpha < 0.05$) between trials were observed.

PIH	%RF of PVF ^a		%RF of VI ^b		%RF of ST ^a	
	Baseline	Meloxicam	Baseline	Meloxicam	Baseline	Meloxicam
0	49.42 $\pm$ 1.69	48.69 $\pm$ 3.31	50.12 (48.31-51.42)	49.72 (47.59-50.50)	50.31 $\pm$ 1.09	49.70 $\pm$ 1.28
8	47.94 $\pm$ 2.39	48.73 $\pm$ 3.47	49.15 (48.13-49.58)	48.68 (48.05-50.77)	50.05 $\pm$ 1.16	50.14 $\pm$ 1.19
24	49.42 $\pm$ 2.20	49.37 $\pm$ 3.27	49.23 (48.22-50.07)	49.49 (48.13-51.24)	49.92 $\pm$ 1.06	50.48 $\pm$ 0.69

^a Normally distributed data shown as mean $\pm$ standard deviation

^b Non-normally distributed data shown as median (interquartile range).

Table 4 Synovial fluid total nucleated cell counts (TNCC, $\times 10^3/\mu\text{L}$), total protein (TP, g/L) and concentration of prostaglandin E₂ (PGE₂, pg/mL) at post-injection hour (PIH) 0, 8, 24 and 48 for both trials (baseline and meloxicam).

PIH	TNCC ($\times 10^3/\mu\text{L}$) ^a		TP (g/L) ^a		PGE ₂ (pg/mL) ^b	
	Baseline	Meloxicam	Baseline	Meloxicam	Baseline	Meloxicam
0	0.25 $\pm$ 0.16	0.24 $\pm$ 0.11	18.0 $\pm$ 3.38	17.3 $\pm$ 2.12	132.90 (98.31-177.94)	96.35 (70.96-115.08)
8	126.6 $\pm$ 21.8	143.7 $\pm$ 21.9	51.5 $\pm$ 6.7	56.8 $\pm$ 4.3	8742.57 (4892.46-15353.79) ^c	620.46 (503.13-1090.80) ^c
24	47.4 $\pm$ 17.6	47.1 $\pm$ 13.2	50.3 $\pm$ 7.1	54.3 $\pm$ 3.9	1318.15 (894.04-1692.53) ^c	401.39 (263.40-636.79) ^c
48	6.25 $\pm$ 2.82	7.35 $\pm$ 2.5	28.5 $\pm$ 2.6	32.0 $\pm$ 4.7	366.49 (299.80-432.43)	202.41 (184.79-264.81)

^a Normally distributed data shown as mean $\pm$ standard deviation

^b Non-normally distributed data shown as median (interquartile range)

^c $\alpha < 0.05$ within time points ($\alpha < 0.05$).

Fig. 1. Mean $\pm$ standard deviation vector sum (mm) measured with the inertial sensor system data (Equinosis Q with Lameness Locator software²) after lipopolysaccharide (LPS) injection, showing the lameness pattern for the baseline trial (red) compared to the meloxicam trial (green). Within time points, statistically significant differences between trials are indicated with * ($\alpha < 0.05$).

Fig. 2. Mean $\pm$ standard deviation synovial fluid total nucleated cell count (TNCC, $\times 10^3/\mu\text{L}$; A) and total protein (TP, g/L; B) for the baseline trial (red) and the meloxicam trial (green). Within time points, no significant differences between trials were observed.

Fig. 3. Median $\pm$ interquartile range of the synovial fluid prostaglandin E_2 (PGE_2) concentration for the baseline trial (red) and the meloxicam trial (green). Within time points, statistically significant differences between trials are indicated with * ($\alpha < 0.05$).