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Effects of intravenous flunixin meglumine, phenylbutazone, and acupuncture on ocular pain scores in the horse: a pilot study

--Manuscript Draft--

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Corresponding Author:	Zita Makra University of Veterinary Medicine: Allatorvostudomanyi Egyetem HUNGARY
First Author:	Zita Makra
Order of Authors:	Zita Makra
	Nóra Csereklye
	Marian Matas Riera
	Richard McMullen
	Kata Veres-Nyéki
Abstract:	In this controlled, blinded, randomized block pilot study the main objective was to evaluate the effectiveness of intravenous flunixin meglumine, phenylbutazone, and acupuncture on ocular pain relief using a multifactorial pain scale in the horse. Four experimental horses underwent corneal epithelial debridement in four sessions, when a randomly selected treatment or a control was used. All horses were pain-scored prior to corneal wounding, then at 18 time points, when 11 parameters were allocated. Differences in the area under the curve of pain scores between the treatment groups were analysed using a paired t-test. Corneal pain was significantly reduced by the third postoperative day (P= .03) when all 11 parameters were considered. Five ocular signs showed significant differences between treatments and proved to be good indicators of ocular pain. The other parameters (heart rate, corneal touch threshold, respond to palpation, and three behavioural parameters) were determined to be irrelevant when evaluating the degree of pain. When considering the five ocular signs, the lowest pain score was attributed to the flunixin meglumine group (1114), followed by the electroacupuncture group (1356), the phenylbutazone group (1397), and the control group (1580). There were significantly lower pain scores (P= .01) in the flunixin meglumine group when compared to those recorded in the control group during the first 46 hours. Flunixin meglumine was the most effective treatment at reducing ocular pain in the horse. In the future, a reduction in the number of pain-score parameters and more precisely defined image evaluation criteria could be utilized.
Suggested Reviewers:	
Opposed Reviewers:	
Response to Reviewers:	

Cover Letter

We would like to submit the following paper entitled 'EFFECTS OF INTRAVENOUS FLUNIXIN MEGLUMINE, PHENYLBUTAZONE, AND ACUPUNCTURE ON OCULAR PAIN SCORES IN THE HORSE: A PILOT STUDY' for publication in Journal of Equine Veterinary Science. Five authors (Zita Makra, Nóra Csereklye, Marian Matas Riera, Richard J. McMullen Jr and Kata Veres-Nyéki) have been involved in the preparation of this manuscript and all of them agreed on its content prior to submission. We declare no conflict of interest. The authors certify that this manuscript is original and not currently under review in any other journal.

This controlled, blinded, randomized block pilot study, to our knowledge, is the first to evaluate the effects of intravenous flunixin meglumine, phenylbutazone and electroacupuncture on ocular pain. An experimental corneal wounding was utilised to create a standard acute ocular pain model, which was then graded using a multifactorial ocular pain scale in the horse. In addition to the first author (ZM), pain scores were recorded by three independent and masked observers based on their assessment of digital clinical images.
We hope that the results and clinical relevance of this study are substantial for you to consider.

Budapest, 27th September 2020

Zita Makra

Reply to the editor's and reviewers' comments

Dear Dr. Edward Squires, Editor-in-Chief and Reviewers,

Thank you very much for evaluating our manuscript JEVS-D-20-00392 entitled "Effects of intravenous flunixin meglumine, phenylbutazone, and acupuncture on ocular pain scores in the horse: a pilot study" and inviting us to resubmit our paper following major revision. In the revised manuscript we have addressed all the comments and revised the manuscript according to your suggestions. We believe the manuscript has improved substantially and we thank all the reviewers and editors for their input and thoroughness.

We have responded to all the reviewer's comments and concerns. Hereafter, we explicitly indicate how we addressed both reviewer's observations.

"A" stands for answer of the authors.
"NL" indicates line number in the new manuscript (file name: Revised_Main_document)
The corrections in the text are highlighted.

Reviewers' comments to author

Reviewer 1

"It is not clear how many observations (eyes) were included into treatment groups. As I understand each horse was treated four times (each eye twice) - 4 observation for animal, 16 totally. Is it correct? Each horse has the same treatment for per session or for all treatment?"

A: Four horses were included into the study. At one session one treatment was selected randomly and only one eye was treated. At one session all 4 horses got the same treatment. Next time 4 weeks later another treatment was selected, and the contralateral eye was treated. So before corneal wounding (t0) and 4 hours later (t1), then 6 hours later (t2) and so on until the end of the 5th day (t18) all horses and treated eyes were observed and scored, so at a treatment session a horse got pain scores from 11 parameters from 1 observer and plus scores from other 3 observers evaluating only the 5 ocular signs that were visible from images. Yes, we had 4 observations in 4 subjects at 18 timepoints.

We corrected the text for better understanding as follows:

NL: "In one session each horse received the same, randomly selected postoperative treatment (flunixin meglumine, phenylbutazone, EA or control)." (L: 86-87)
"From the eleven parameters only five ocular signs (blepharospasm, tearing, eyelid swelling, corneal opacity, conjunctival hyperaemia/chemosis) were scored by the ophthalmologists, when they evaluated the clinical images according to the pain scale described above." (L: 142-146)

The control group included horses with corneal wounds treated by saline (placebo). It is acceptable for treatment methods comparison. However, in this study the ocular pain scoring system is not widely accepted or gold standard method but the developed by authors exactly for this study. Thus, control group of healthy horses should be added at least.

A: That is why we included 4 sessions, 3 with treatments and another with a placebo control, as a Control group. In a healthy horse or eye the pain score is zero or minimal, so that we could compare the analgetic effect of a selected drug to the control in a horse.

In my opinion, the first step should be evaluation of ocular pain scoring system itself. The behavior, face rubbing, heart rate, corneal touch, response for palpation etc. may differs between individual healthy horses.

A: Yes, they may differ, but in healthy horses these parameters should be within normal limits (not painful), so pain score can be 0. Painful, diseased eyes can be compered to each other, but in this situation the underlying infection, type of ulceration, degree of inflammation, individual pain threshold may influence the pain reactions.

Groups of healthy horses and sick horses with different eye diseases - big enough to obtain normal data distribution should be investigated before.

A: Our main goal was to compare the analgesic effect of flunixin, acupuncture and phenylbutazone, and for this purpose we used a composed pain scale.

The pain scoring during the study were measured by one observer only and not blinded. Two others observers evaluated only photos. Thus, they were not able to evaluate pain score.

A: Three other blinded observers gave pain scores for 5 ocular parameters, and these pain scores showed marked reduction over time and best reflected the clinical pain levels. For further comparison between the treatment groups only these parameters were subsequently included and used for statistical analysis.

The whole ocular pain scale or just comprised 5 ocular signs are important ?

A: Originally we wanted to include objective measurable parameters such as HR, CTT and behavioral factors, and it is true that they were evaluated by a single observer who was present at the study, but some of these parameters didn't changed or changed inconsistently. Only the ocular signs changed reliably that can reflect the comfort level. This was the reason that we used the pain scores from the 5 ocular signs for treatments comparison, so from this viewpoint only the 5 ocular signs were important.

Thus, I do not agree with author's statement regarding ocular pain scoring system for horses development.
Four observation per group is to small sample for proper analysis. Moreover, two horses from control group got rescue analgesia. Thus, how much observations were finally used for statistical analysis?

A: Yes, the sample size was low, it was a pilot study, but we think, that at one timepoint 4 observers opinions were enough to evaluate the degree of pain. Eyes were scored at each treatment (4 sessions) by 4 observers at 18 timepoints and 5 parameters were considered to compere the treatment groups.

The statistical analysis by paired-sample t-test for 4 observation in the group (less in control) is not proper method. Also, I do not understand how AUC was calculated. According the statistical analysis method (line 165) it was done for aggregated score in group.

A: We wanted to compare the pain scores of treatments, so we calculated the AUC (with the software and graphs-Figure2) in order to eliminate individual aspects of the horses and not to grab

only time points and to analyze the whole time period of the study from the postop 4 hrs up to 106 hrs. For better understanding we changed the text as follows:

NL: "The following statistical program was used: R 3.2.2 (R Core Team 2019, R Statistical Computing, Vienna, Austria, https://www.R-project.org/). The area under the curve (AUC) was used as an aggregated pain score of horses in the different treatment groups. AUC in the control and treatment groups were calculated with the software and control was compared to treatment groups using paired-sample t-test." (L: 165-169)

However, according the results (line 210) for individual horse. What was a golden standard here?

A: We corrected it as follows:

NL: "*Table 2* shows pain scores from the five ocular parameters in horses from each treatment group. *Figure 3* shows the graphs of aggregated pain scores from 5 ocular parameters. The area under the curve was calculated for the individual horses in all treatment groups. The differences in AUC of the control and treatments of a horse was calculated, the means of differences of the 4 horses compared using paired-sample t-test, null hypothesis: H0= mean of differences is equal to 0. Greater pain scores were expected to be associated with horses in the control group. Significant differences were found only between the control and flunixin groups during the first 46 hours of the study, since control $AUC_{0\text{-}46}$ was 376.08 and flunixin $AUC_{0\text{-}46}$ was 317.0 (P= .01). The pain scores were consistently higher in the control group compared to all of the other treatment groups, but the differences were not statistically significant." (L: 206-216)

The data provides in lines 204 - 209 are the same as in table 1.

A: Yes, we deleted these data from the text.

The bias may be huge during analysis of such small groups.

A: One of the limitations was the small sample size, but we did not mentioned it in the text, because this was a pilot study.

Figures 1, 2 contain pain score and pain score from 5 parameters. Both shown, the same tendency.

A: This also shows that the irrelevant parameters do not affect the pain curve much. That is why we focused on the relevant 5 ocular parameters in the statistical analysis. It is also evident that the differences between treatments are more prominent in the first 46hrs.

Figure 4 shows, that horse no 4 suffer high pain despite the flunixin treatment. So, this treatment didn't work in 25% of treated animals and should not be considered as better treatment than other methods.

A: Horse4 showed high pain in the flunixin and in the phenylbutazone treatment and he looked to be the most sensitive horse having the highest total pain scores. (Table2) We could not find explanation why H4 and H1 had score peaks within the first 2 days of the study in this 2 treatment groups. But the tendency of pain score reduction was very similar in all horses, so we could conclude that none of them was more or less sensitive compared to the others in any of the treatment groups throughout the duration of the study (Figure4). One peak in pain scores in a horse does not mean that the means of AUC differences of the 4 horses can not differ significantly between the control and flunixin groups during the first 46 hours of the study. All pain scores were

consistently higher in the control group compared to all of the other treatments, but the differences were not statistically significant.

How looks individuals score parameters in each horse?

A: Table2 and Figure4 contains combined pain scores from the five ocular parameters for each horse and treatment group.

I agree with most author's conclusion excluding flunixin efficacy.

Reviewer 2

Introduction

Page 1 Line 13-When considering the five ocular signs, the lowest pain score was attributed to the flunixin meglumine group (1114), followed by the electroacupuncture group (1356), the phenylbutazone group (1397), and the control group (1580).

NL: "When considering the five ocular signs, the lowest pain score was attributed to the flunixin meglumine group (1114), **followed by** the electroacupuncture group (1356), the phenylbutazone group (1397), and the control group (1580)." (L: 13-16)

Methods

-concerns about the masked observers judging clinical images

-more sensitive to photography as the process goes on

A: The experienced observers were masked to the treatment and the images about horses were sent also in random order to them, so we think they evaluated and scored the clinical signs objectively from the beginning till the end of the study course.

-any comparison between order of treatments?

-do subsequent ulcers heal more slowly, especially since two horses developed long-term corneal fibrosis, can the cornea really be back to normal within 4 weeks

A: We could not observe slower healing time at the case of second wounding of an eye. The corneal fibrosis in the 2 horses was very minimal, not visible with inspection, just with great magnification using 16x magnification oculars at slit-lamp biomicroscopy. Maybe the basal membrane and some superficial collagen fibers were also removed with the wounding, so new collagen fibers could not be reorganized perfectly to provide excellent transparency.

NL: "Two horses retained an extremely faint superficial corneal opacity within the axial cornea (site of corneal wounding) at 1.5 years postoperatively, which was only visible with careful slit-lamp biomicroscopic examination." (L: 190-192)

-phenylbutazone can be given q12h, is there a reason why q24h was chosen

A: According to the manufacturer's instructions phenylbutazone at dose of 4.4 mg/kg, administered intravenously, is recommended to give once a day. Maybe in great pain, such as bone fracture, the dose can be doubled in a day, but in our study horses had mild pain, we expected mild-moderate pain after wounding so we took the manufacturer's instruction into account.

Results/Discussion

Page 8 Line 185- This sentence runs on, please correct.- All corneal wounds were completely healed,the corneal healing time, as noted by negative fluorescein uptake, was 5 days in most of the cases (87.5%, 4–12 days), regardless of the treatment group.

NL: "All corneal wounds were healed, as noted by negative fluorescein uptake, within an average of 5 days." (L: 187-188)

-reason for using CTT despite reported variation with humidity (Lum E, Murphy PJ. Effects of ambient humidity on the Cochet-Bonnet aesthesiometer. Eye. 2018 Oct;32(10):1644-1651.)

A: To evaluate corneal sensitivity we wanted to include an objective parameter, such as CTT, because still this measurement is considered the most reliable esthesiometry technique according to the equine literature, although there are many influencing factors: ambient humidity, the condition of the thread, experience of the observer, horse temperament.

Highlights

– This is the first report of an ocular pain scoring system being used in horses.

– The manual debridement of corneal epithelium and basement membrane in horses was found to be a reliable and safe model to induce acute ocular pain.

– Ocular pain peaked within 2-3 days after corneal wounding in the present study.

– Flunixin meglumine appears to more reliably ameliorate corneal pain in the horse, when compared to phenylbutazone or electroacupuncture.

ABSTRACT

In this controlled, blinded, randomized block pilot study the main objective was to evaluate the effectiveness of intravenous flunixin meglumine, phenylbutazone, and acupuncture on ocular pain relief using a multifactorial pain scale in the horse. Four experimental horses underwent corneal epithelial debridement in four sessions, when a randomly selected treatment or a control was used. All horses were pain-scored prior to corneal wounding, then at 18 time points, when 11 parameters were allocated. Differences in the area under the curve of pain scores between the treatment groups were analysed using a paired t-test. Corneal pain was significantly reduced by the third postoperative day (P= .03) when all 11 parameters were considered. Five ocular signs showed significant differences between treatments and proved to be good indicators of ocular pain. The other parameters (heart rate, corneal touch threshold, respond to palpation, and three behavioural parameters) were determined to be irrelevant when evaluating the degree of pain. When considering the five ocular signs, the lowest pain score was attributed to the flunixin meglumine group (1114), **followed by** the electroacupuncture group (1356), the phenylbutazone group (1397), and the control group (1580). There were significantly lower pain scores (P= .01) in the flunixin meglumine group when compared to those recorded in the control group during the first 46 hours.

Flunixin meglumine was the most effective treatment at reducing ocular pain in the horse. In the future, a reduction in the number of pain-score parameters and more precisely defined image evaluation criteria could be utilized.

Keywords *cornea, electroacupuncture, pain score, nonsteroidal anti-inflammatory drug*

INTRODUCTION

Ocular trauma and corneal ulcers are common problems in horses, and result in varying degrees of ocular discomfort. Horses with superficial ulcers generally exhibit significant discomfort owing to the extensive sensory innervation of the superficial cornea [1]. Much of the cornea's sensory innervation is achieved via the ophthalmic branch of the trigeminal nerve (cranial nerve V, CN V). Ciliary nerve branches enter the limbus at the level of the midstroma and course toward the superficial cornea. Free endings of sensory nerves are found at the wing cell level of the epithelium, which makes the cornea one of the most sensitive tissues in the body[2]. Corneal damage results primarily in inflammatory pain, resulting in the routine use of nonsteroidal anti-inflammatory drugs (NSAID) in addition to specific etiological treatment [1].

Phenylbutazone and flunixin meglumine are the preferred NSAIDs used to treat orthopaedic or colic cases in equine practice [3, 4]. Despite having not yet been evaluated for clinical efficacy, NSAIDs are widely used to treat ocular pain in horses. Furthermore, there are currently no established standards to evaluate ocular pain in horses under clinical conditions. Recently, multifactorial pain scales have become more commonly used to evaluate pain in clinical patients with orthopaedic or abdominal pain [5, 6, 7, 8, 9]. Ideal pain scales can indicate the severity of the pain and therefore facilitate the documentation of the response to specific treatment. Composing our pain scale, we combined the composite pain scale published by Bussieres et al [5] by replacing lameness-related indices with eye-related factors with an ocular exam scoring system for dogs used by Clark in 2011 [10].

The application of acupuncture (AC) and electroacupuncture (EA) in veterinary practice is relatively new in Europe [11, 12]. The use of AC to facilitate a reduction in intraocular pressure [13], and to provoke analgesia in horses with equine recurrent uveitis, has been reported [14]. According to the principles of Traditional Chinese Medicine, AC is

proposed to re-establish the homeostasis of the main organs by modulating the flow of Qi and Xue through the meridians [15]. There is evidence in Western Medicine that acupuncture analgesia is based on endogenous opioid-like substance release and activation of the diffuse noxious inhibitory control system (DNIC) mainly via modulating neurotransmission on the adrenergic, serotonin and glutamate receptors in the central nervous system [16, 17]. Applying low EA frequencies (2Hz) results in increased β-endorphine, enkephalin, endomorphine release targeting the μ and δ opioid receptors, while high EA frequencies (100Hz) facilitate dynorphine release and responsible for λ receptor effects [16, 18]. Therefore, the treatment can be tailored according to specific therapeutic goals [12, 19].

Standardized measures of pain levels are necessary to compare the effects of different analgesic treatments on ocular pain. Our goal was to utilize a model which induces a long-lasting (minimum of 48 hours) pain stimulus allowing assessment of the response to analgesic therapy, without severe long-term consequences such as intraocular inflammation, significant corneal scarring or permanent visual impairment. Corneal epithelial removal should be uniform. The induced pain should be reproducible and characteristic, while remaining moderate in severity. Mechanical epithelial debridement performed with an Algerbrush II (corneal rust ring remover) was chosen as the model of experimentally induced acute ocular pain [10, 20]. The resulting corneal lesion is expected to heal within seven days [1].

This study, to our knowledge, is the first to evaluate the effects of intravenous flunixin meglumine, phenylbutazone and EA on ocular pain caused by experimental corneal wounding to create a standard acute ocular pain model, which was then graded using a multifactorial ocular pain scale, in the horse. In addition to the first author (ZM), pain scores were recorded by three independent and masked observers based on their assessment of

digital clinical images. Our hypothesis was that each analgesic treatment reduces corneal pain.

MATERIALS AND METHODS

Four horses (two Hungarian warmbloods, one Haflinger, and one Thoroughbred) aged between six and nine years of age (two mares and two geldings) owned by the Equine Department and Clinic, University of Veterinary Medicine Budapest, were included into this randomized, block-designed, placebo-controlled study. Complete preoperative ophthalmic examinations confirmed that all of the eyes included in this study were within normal limits. The study received ethical approval from the Animal Care and Use Committee of the University of Veterinary Medicine, Budapest (Reg. No: 11/4/2015). In the present study a 6 mm disc of axial corneal epithelium and its basement membrane was manually removed, alternating between the left and right eyes, on four different occasions with a four-week rest interval between sessions. **In one session** each horse received the same, randomly selected postoperative treatment (flunixin meglumine, phenylbutazone, EA or control). Simple randomization technique was utilized (pulling from hat).

Corneal wounding

Each horse was sedated with detomidine hydrochloride (0.01 mg/kg IV, Cepesedan 10 mg/mL inj. Medicus Partner), and an auriculopalpebral nerve block (1.5 mL lidocaine SC, 2% Lidocain inj. Teva) was peformed on the respective eye. Topical oxybuprocaine (Novesine 0.4 % Augentropfen, OmniVision) was used for topical corneal analgesia. The ocular surface was first irrigated with 0.5% povidone-iodine solution (Betadine, Egis), and then rinsed with saline. The horses's head was supported on a purpose-made stand and the eyelids were held open manually. A 6 mm round, central corneal epithelial and basement

membrane lesion was created with an Algerbrush II (Eickemeyer, Germany) corneal rust ring remover after outlining the wound edge with a corneal trephine (Eickemeyer, Germany). The cornea was stained with fluorescein dye (Fluorescein sodium ophthalmic strips, Eickemeyer) immediately following the procedure and on days 3, 5 and 7. Sodium-hyaluronate was administered topically (Aptus SENTRX Eye Gel, Orion Pharma) post-operatively q8h. Wounds were defined as healed when fluorescein dye was no longer retained, and treatments and pain scoring were then discontinued for that particular horse.

Treatment groups

Flunixin group: 1.1 mg/kg flunixin-meglumine (Flunidol MLS 50mg/mL inj, CP-Pharma) was administered intravenously (IV) 4 h after the corneal wounding followed by the same dose q12h (according to the manufacturer's instructions) until the lesion healed.

Phenylbutazone group: 4.4 mg/kg phenylbutazone (CP-Phenylbutazon 20 % inj. Medicus Partner) IV was administered 4 h after the corneal wounding followed by the same dose q24h until the lesion healed.

EA group: EA treatment was applied for 20 minutes to the stomach meridian #1 (ST1, intersection between the medial and middle one third of the lower eyelid), the gall bladder meridian #1 (GB1, 0.75 cm lateral to the lateral canthus), the bladder meridian #1 (BL1, medial canthus, in the indention dorsal to the base of the nictitating membrane) and the triple heater meridian #23 (TH23, *Jing shu*, eye association point - at the midpoint of the superior eyelid, ventral to the zygomatic process of the frontal bone) acupoints q24h until the lesion healed. *(Figure 1)*

Control group: saline injection (SalsolA 250 mL infusion, Teva) in same volume as phenylbutazone IV q24h.

Electroacupuncture

For EA we used 0.25x30mm long Chinese steel needles (Dongbang AcuPrime) connected to an EA device (AS Super 4 Digital, Pierenkemper) at 4 acupoints around the eye (*ST1, GB1, BL1, TH23) (Figure 1).* A human "Sensitive high frequency" protocol (recommended by the manufacturer for pain treatment of the face in humans) was used (80 Hz, 60 μs), the intensity was gradually increased at the beginning until fine muscle fasciculation was noticed on the eyelid (0.5-1.3 mA), and then this intensity was kept for the 20 minutes long treatment.

Pain scoring

Eleven parameters were evaluated in the multifactorial ocular pain scoring system: heart rate (HR), corneal touch threshold (CTT), six ocular signs and three behavioural parameters *(Table 1)*. All horses were pain scored before corneal wounding in order to establish baseline parameters (T_0). Pain scoring was continued and the first treatment was applied in each horse four hours after corneal wounding after the effects of topical and perineural anaesthesia had worn off [21]. Pain scoring was performed by a single trained observer (ZM) at 4, 6, 8, 10 hours postoperatively, then every 4 hours for the first 2 days and then twice daily through the 5^{th} day ($T_{1\text{-}18}$). The intensity of ocular signs was graded on a 0–3 scale (0: WNL, 1: mild, 2: moderate, 3: severe) (*Table 1*). The eyes were photographed at each time point and sent for evaluation by three independent observers (two board certified ophthalmologists: RJM [DACVO, DECVO], MMR [DECVO], and SzM [DVM]), masked to the treatments. **From the eleven parameters only five ocular signs (blepharospasm, tearing, eyelid swelling, corneal opacity, conjunctival hyperaemia/chemosis) were scored by the ophthalmologists, when they evaluated the clinical images according to the pain scale described above.**

The central area of the cornea was used for both the corneal wounding and CTT measurements, as this area is the most sensitive in healthy horses [2]. The CTT value is inversely related to the filament's length of the esthesiometer. The CTT threshold has been previously defined by evoking a blink reflex in three out of five attempts of direct corneal stimulation (bending of the filament) using a specific length of nylon filament, that is gradually shortened using a Cochet-Bonnet esthesiometer [22]. We cut the long hair (vibrissae) around the eye and utilized the same esthesiometry technique (Luneau Cochet-Bonnet Esthesiometer, Neo-Visus) and compared the values in mm to the baseline value (T_0) [22].

Rescue analgesia

Any horse with a total pain score ≥16 (maximum possible=33) or that scored ≥3 in two or more individual parameters was given rescue analgesia, morphine hydrochloride, 0.1 mg/kg IM (Morphinum hydrocloricum 20mg/mL, Teva).

Statistical analysis

The average pain scores for blepharospasm, tearing, eyelid swelling, corneal opacity and conjunctival hyperaemia, chemosis were calculated for various time points and drawn as a function of time. **The following statistical program was used: R 3.2.2 (R Core Team 2019, R Statistical Computing, Vienna, Austria, https://www.R-project.org/). The area under the curve (AUC) was used as an aggregated pain score of horses in the different treatment groups. AUC in the control and treatment groups were calculated with the software and control was compared to treatment groups using paired-sample t-test.** Paired-sample t-test was also used to determine if there was a decrease in pain scores over time. Significance was inferred at $P \leq .05$.

RESULTS

Changes of pain scores over time

Average pain scores for all 11 parameters were calculated at various time points for each horse and the results combined according to the individual treatment groups. The changes in mean pain scores over time are illustrated in *Figure 2.*

Pain scores at T_0 were uniformly 0, representing the lack of detectable discomfort or pain. The pain-scoring started at 4 hrs (T_1) and ended at 106 hrs (T_{18}). Based on the diagram (*Figure 2*) the mean total pain scores decreased appreciably by the end of the study (T_{18}= 106 hrs postop). There was a significant decrease between the mean pain scores from the first day (between 4-22 hrs), and those recorded on the last day (between 82-106 hrs). The mean difference in pain score reduction between the first and fifth days was 7.29 points (P= .01). The maximum pain score never reached a value of 11 in the treatment groups, representing a mild level of pain. In the control group, the maximum pain score was 12, representing a mild-moderate level of pain.

All corneal wounds were healed, as noted by negative fluorescein uptake, within an average of 5 days. None of the eyes became infected or demonstrated any other severe complication. **Two horses retained an extremely faint superficial corneal opacity within the axial cornea (site of corneal wounding) at 1.5 years postoperatively, which was only visible with careful slit-lamp biomicroscopic examination.**

Pain-score variations between different parameters for each group over time

Behavioural parameters (comfort, appetite, head rubbing) changed the least, with mean pain scores never exceeding 0.5 points in any treatment group. HR changes were also minimal, highest score was 1 point. There was a large degree of inter- and intrasubject variation in

CTT measurements. Response to palpation of the adnexa was inconsistent and pain scores for this parameter varied greatly over time. The pain scores for the five ocular signs (blepharospasm, tearing, eyelid swelling, corneal oedema, conjunctival hyperaemia/chemosis) showed marked reduction over time and best reflected the clinical pain levels. For further comparison between the treatment groups only these parameters were subsequently included. Pain scores for blepharospasm varied the most in each horse (0-3 points) and correlated well with the severity of pain over time.

Pain scores of treatment groups

Table 2 **shows pain scores from the five ocular parameters in horses from each treatment group.** ***Figure 3*** **shows the graphs of aggregated pain scores from 5 ocular parameters. The area under the curve was calculated for the individual horses in all treatment groups. The differences in AUC of the control and treatments of a horse was calculated, the means of differences of the 4 horses compared using paired-sample t-test, null hypothesis: H0= mean of differences is equal to 0. Greater pain scores were expected to be associated with horses in the control group. Significant differences were found only between the control and flunixin groups during the first 46 hours of the study, since control $AUC_{0\text{-}46}$ was 376.08 and flunixin $AUC_{0\text{-}46}$ was 317.0 (P= .01). The pain scores were consistently higher in the control group compared to all of the other treatment groups, but the differences were not statistically significant.**

Pain scores in individual horses

Pain scores for individual horses comprised of the five previously described ocular parameters varied the most throughout the study. The mean individual pain scores for each horse within the different treatments groups over time can be seen in *Figure 4.*

Rescue analgesia

The necessity for morphine administration (0.1 mg/kg, IM) as a rescue analgesic was a component of the definition of treatment failure in the present study. Horses receiving rescue analgesia still underwent pain scoring and continued topical sodium-hyaluronate administration until the corneal wounds were healed. Two horses from the control group received pain scores of 3 in two or three individual categories and were administered rescue analgesia. Horse2 received an IM injection of morphine once at T_3 (8 h postoperatively) as she received 3 points for blepharospasm and conjunctival hyperaemia/chemosis. Despite Horse2 receiving the same scores at T_4 (10 h postoperatively), she did not receive a second IM injection, as the dose received at T_3 should provide analgesia for up to six hours [23]. Horse4 received IM morphine twice. First, after receiving three points for blepharospasm and conjunctival hyperaemia at T_2 (6 h postop). Although, his scores didn't change at T_3, T_4, T_5, T_6, the IM morphine injection was repeated at T_5 (14 h postop). This horse also received a score of 3 points for eyelid swelling, too.

DISCUSSION

The manual debridement of corneal epithelium and basement membrane in horses was found to be a reliable and safe model to induce acute ocular pain in our study. None of the horses had corneal neovascularisation and all of the corneal lesions reepithelized. Based on our pain scale, mild to moderate pain was induced, because the total pain score at any time point was lower than 14 (*Table 1*: max. point: 33, mild pain: <11, moderate pain: >11, severe pain: >22), but clinical signs remained present throughout the duration of the evaluation period.

In humans, mean pain scores decreased significantly by 72 hrs postoperatively [24], whereas in dogs mean pain scores returned to baseline values by 48 hrs [10]. Considering

values from similar time points in our study, the mean decrease in pain scores was 2.92 points by 46 hrs (P= .07), which represents 40.5% of the total decrease, and 4.09 points by 70 hrs (P= .03), representing 56.1% of the total pain score decrease. Subsequently, the reduction in total pain score was significant by the 3rd day postoperatively. Our pain score results support the importance of early supplemental analgesia in the presence of corneal ulceration, as the horses in this experimental study experienced the greatest degree of pain during the first three days following corneal wounding.

A limitation of this study is the inconsistent pain score values generated from HR, CTT, and response to palpation of adnexa. Another limitation was the limited value of the three behavioural parameters (comfort, appetite, head rubbing). Scores greater than 0 were only occasionally assigned. The reason for these consistently low scores may have been associated with the level of the pain was not great enough to visibly affect the horse. The ocular signs evaluated by the masked observers more accurately reflect the variability between the treatment groups and proved valuable in distinguishing between low and moderate levels of pain intensity. In the present study, blepharospasm proved to be the most consistent indication of pain demonstrating wide variability in its pain scores (ranging from 0–3). Despite consistent environmental factors amongst all of the treatment groups (same stall, feed, temperature, nursing personnel, etc.), habituation to treatment may have influenced pain score values. A crossover study design might have eliminated possible modifying factors such as habituation or time. However, only two different medications were applied in each eye with a two-month wash-out period between treatment phases. The influence of environmental factors or habituation to pain, such as topical or systemic treatment or clinical photography couldn't be completely discounted, so pain scores between treatment groups were compared to control horses. Clinically effective ocular pain relief was documented in all three treatment groups when compared with the control group. However,

the only significant difference was found between the flunixin and placebo groups (P= .01) during the first 46 hours. Based on these results, flunixin meglumine should be used preferentially over phenylbutazone or EA to manage corneal pain.

To date, and to the best of our knowledge, this is the first report of an ocular pain scoring system being used in horses. Our main objective was to include measurable categories and use an objective scoring system to grade ocular exam findings. Trained observers, who were masked to the treatments, scored the ocular signs based on photographs. In future studies, parameters such as HR, CTT, behavioural changes, response to palpation of adnexa can be eliminated from the pain scoring system, as they could neither be consistently nor reliably associated with ocular pain.

Individual expression of discomfort (pain) was confined to certain, short durations of time that varied from horse to horse. Horse4 had its maximum pain score while in the flunixin group. But, both Horse1 and Horse4 had elevated scores while in the phenylbutazone group, for which we couldn't find any explanation. Clinically, Horse3 showed the mildest pain signs (i.e., lowest pain scores), and her corneas healed the fastest (cornea became fluorescein negative by the 4th day). None of the horses was more or less sensitive compared to the others in any of the treatment groups throughout the duration of the study.

Several studies have demonstrated that, even with appropriate scoring systems, control groups may not always have a 100% rescue rate [10, 25]. In the present study, rescue analgesia was only necessary for two horses in the control group. Pain scores never reached 16 points (50% of the total possible score), which was the second criteria requiring the application of rescue analgesia. In this study IM injections of morphine (rescue analgesia) failed to ameliorate ocular pain. Better analgesia was achieved in the flunixin meglumine, phenylbutazone, and EA groups compared to the control group. Rescue analgesia was

required three times in this study in two horses in the control group. In a recent study in dogs, rescue analgesia was required for subjects in the treatment groups, as well [10]. Corneal wounding incites local inflammatory reactions (oedema, conjunctival hyperaemia) which result in ocular pain. Opioids are analgesic and modulate pain sensation, but do not reduce inflammation. In the control horses receiving rescue analgesia the clinical signs remained and the pain scores recorded after IM injection of morphine did not reflect an attenuation of pain. Furthermore, a study evaluating the use of topical ophthalmic 1% morphine sulphate did not lead to an increase in the corneal touch threshold of intact and healthy equine corneas [26]. To date, there have been no studies evaluating intramuscular injection of morphine on ocular pain relief.

In conclusion, flunixin meglumine appears to more reliably ameliorate corneal pain, when compared to phenylbutazone or EA. Ocular pain peaked within 2-3 days after corneal wounding in the present study in horses, highlighting the importance of appropriate analgesic management in the presence of corneal ulcers. Our clinical pilot study confirms that responses to pain stimuli are individual and evaluation of pain sensation in animals is still challenging. The ocular pain scoring system, after further refinement, can be used for further analgesic comparisons and in clinical studies.

List of figure legends

Figure 1. Electroacupuncture acupoints around the eye demonstrated on Horse1. Stomach meridian #1 (ST1), gall bladder meridian #1 (GB1), bladder meridian #1 (BL1), and triple heater meridian #23 (TH23).

Figure 2. Mean total pain scores of treatment groups over time

Figure 3. Mean pain scores for each treatment group from the five ocular parameters over time.

Figure 4. Mean pain scores for individual horses, comprised of the five ocular signs in the different treatment groups over time. X axis: postoperative time in hours, Y axis: mean pain scores

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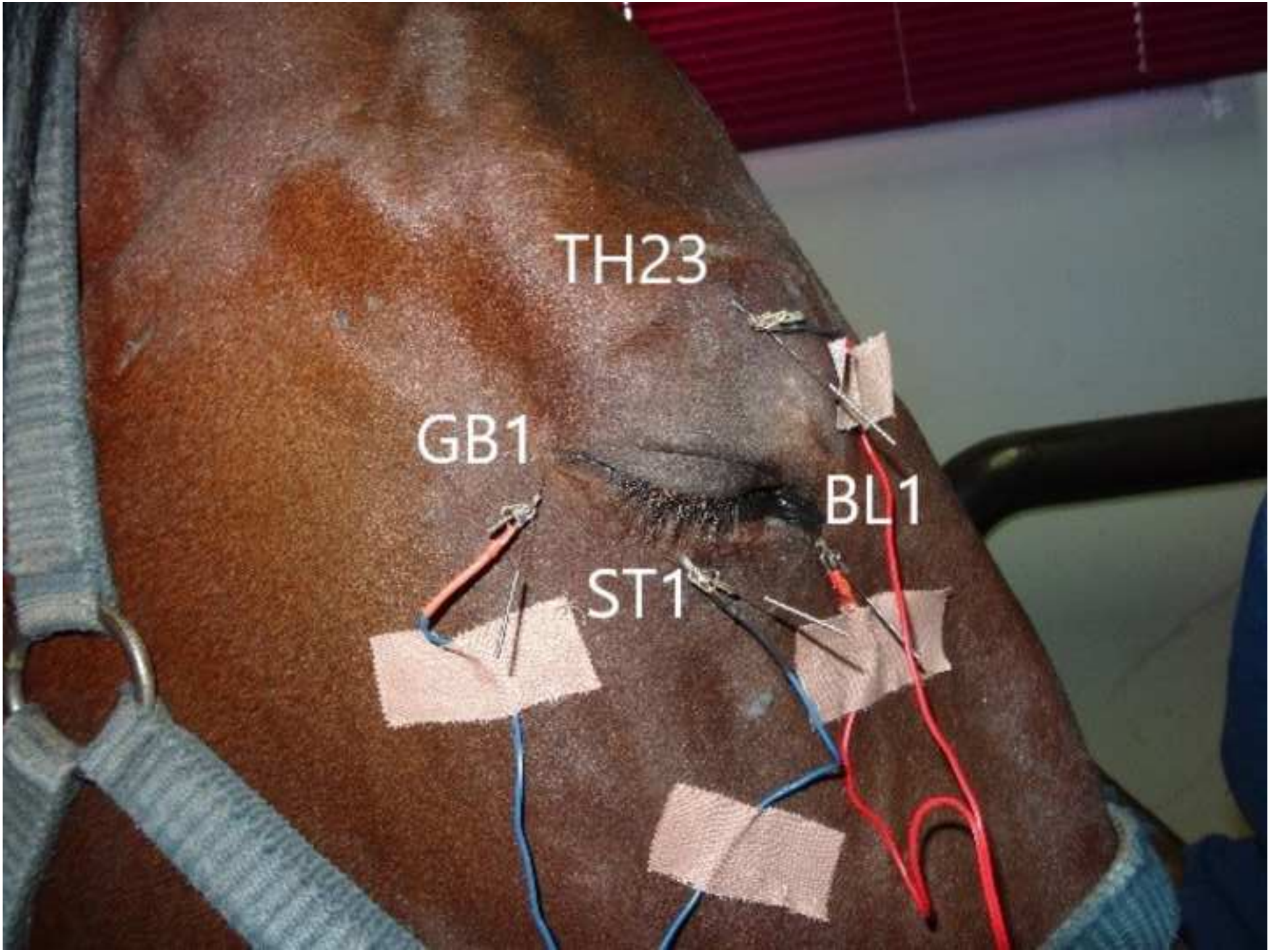

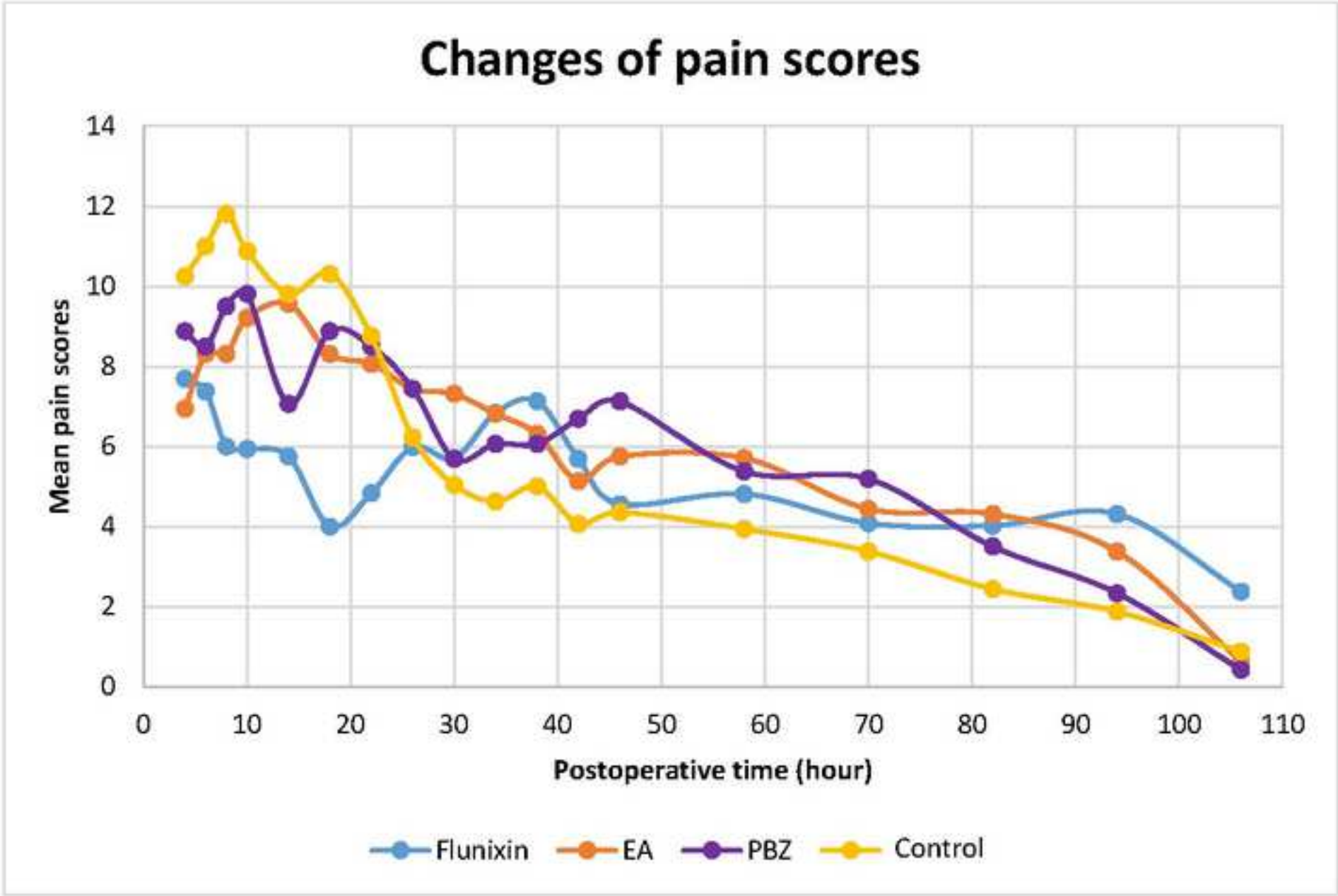

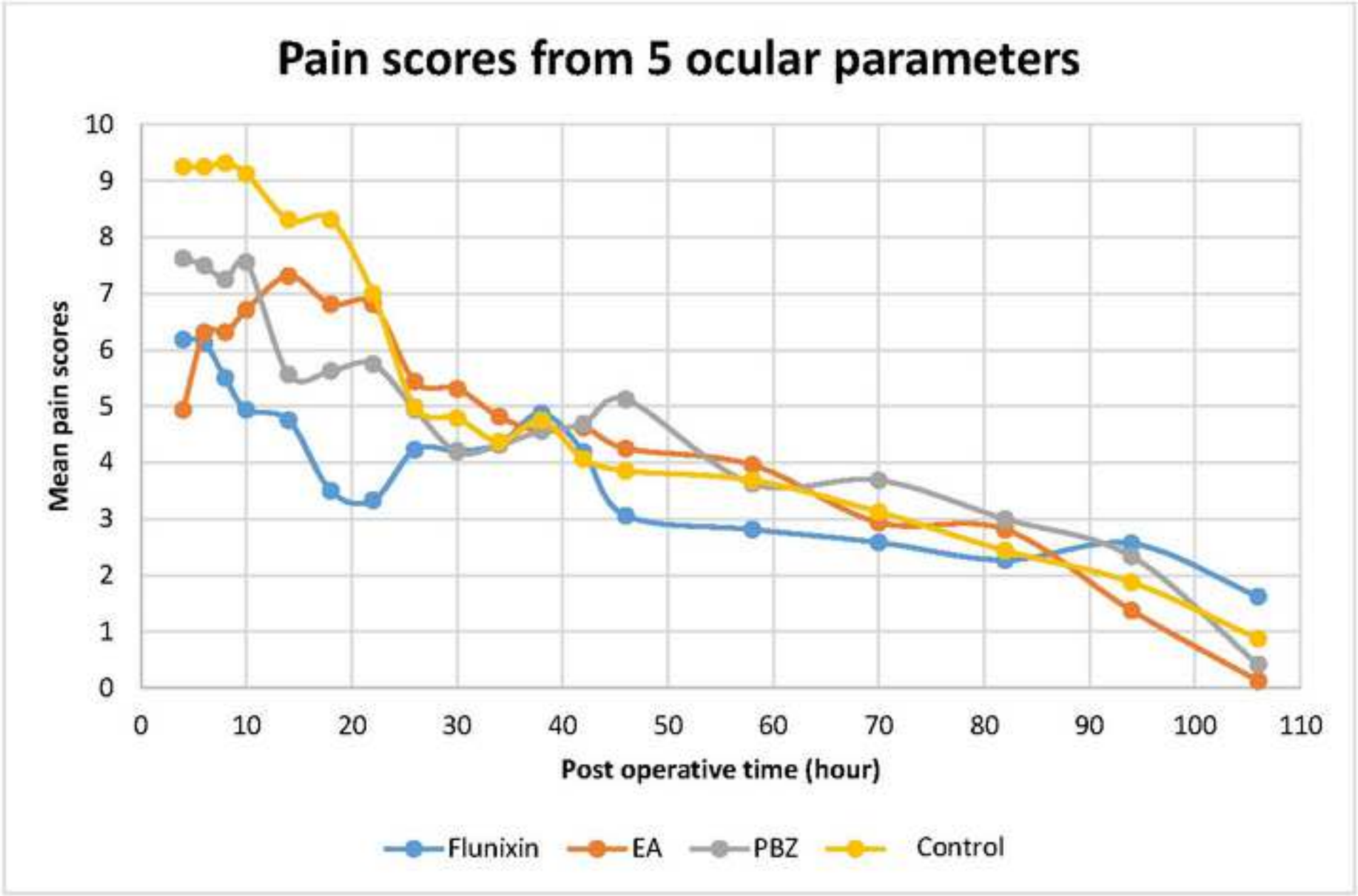

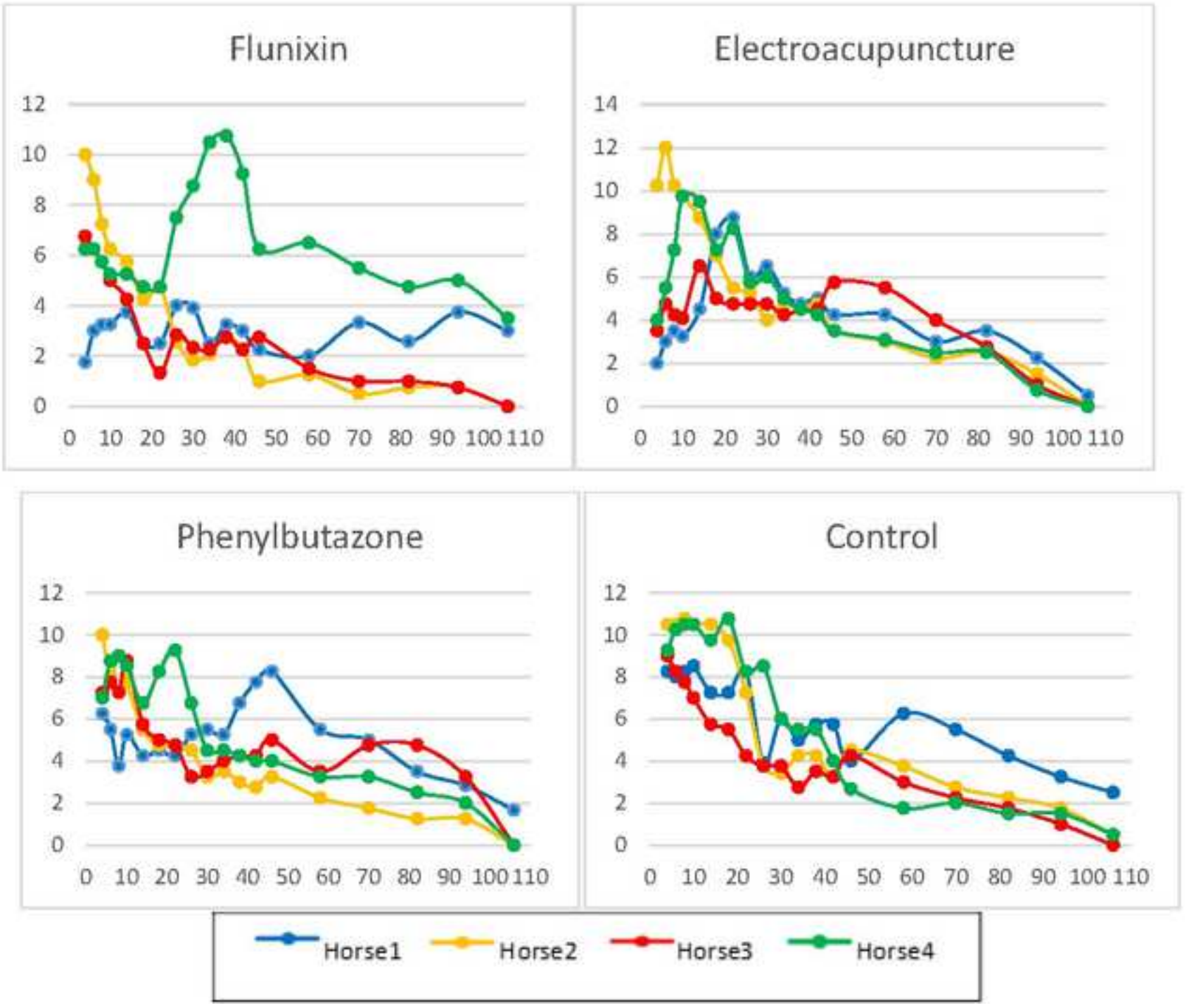

Effects of intravenous flunixin meglumine, phenylbutazone, and acupuncture on ocular pain scores in the horse: a pilot study

Zita Makra[1], Nóra Csereklye[1], Marian Matas Riera[2], Richard J. McMullen Jr[3], Kata Veres-Nyéki[4]

[1]Equine Department and Clinic, University of Veterinary Medicine Budapest, Hungary

[2]Memvet Oftalmologia, Palma, Mallorca, Spain

[3]Auburn University, JT Vaughan Large Animal Teaching Hospital, Auburn, Alabama, USA

[4]Royal Veterinary College, Anaesthesia and Analgesia Service, London, UK

Corresponding author: Zita Makra

e-mail: makra.zita@univet.hu

Address: University of Veterinary Medicine, Department and Clinic of Equine Medicine, Budapest Hungary,

Postal address: Equine Clinic, Üllő, 2225, Doramajor, Hungary

Authors' contributions

Z. Makra contributed to study design and implementation, data interpretation, and manuscript preparation.

N. Csereklye contributed to study implementation and manuscript preparation.

M. M. Riera contributed to study implementation and manuscript preparation.

R. J. McMullen contributed to study implementation and manuscript preparation.

K. Veres-Nyéki contributed to study design and manuscript preparation.

All authors contributed to and approved the final manuscript.

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Conflict of interest

No competing interest have been declared.

Ethical Animal Research

This study was approved by the Institution Animal Care and Use Committee at the University of Veterinary Medicine, Budapest (Reg. No: 11/4/2015).

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[1]Equine Department and Clinic, University of Veterinary Medicine Budapest, Hungary

[2]Memvet Oftalmologia, Palma, Mallorca, Spain

[3]Auburn University, JT Vaughan Large Animal Teaching Hospital, Auburn, Alabama, USA

[4]Royal Veterinary College, Anaesthesia and Analgesia Service, London, UK

Corresponding author: Zita Makra

e-mail: makra.zita@univet.hu

Address: University of Veterinary Medicine, Department and Clinic of Equine Medicine, Budapest Hungary,

Postal address: Equine Clinic, Üllő, 2225, Doramajor, Hungary

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