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Behavioral Responses to Touch and Leading in Lippizan Mares: Natural vs. Traditional Handling Method

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ABSTRACT

Background: Proper handling and a well-established human-animal relationship influence both the behavior and the welfare of an animal.

Methods: In this study, we compared the behavioral responses to touch and leading in mares using two handling methods. A traditional method (T) was based on forced cooperation and a natural (TTEAM) on tactile stimulation and mares' natural behavior. Twenty-two group housed Lipizzan mares participated. Throughout four consecutive weeks, there were six observational days per method and based on a predefined ethogram, mares were scored daily on a 6-point scale. Higher scores indicated better reception of touch and leading, relaxation and cooperation, whereas lower scores indicated agonistic and/or fear-related behaviors. The time spent to implement touch and leading was also recorded.

Result: Despite significant individual differences, touch and leading scores were found to be higher in the TTEAM compared to the T. Our results imply that mares were more relaxed, displayed better cooperation and had reduced agonistic and fear-related behavior during TTEAM. We suggest that implementation of TTEAM positively affected mares' reception of touch and leading, but due to variation in behavioral plasticity, the importance of mare's individuality is accentuated.

Key words: Cooperation, Horses, Individual differences, Natural horsemanship.

INTRODUCTION

Horse-human interaction is a big part of everyday life of a horse (Kelly *et al*., 2021). Methods of keeping and managing domesticated horses are mainly based on traditional methods, which often involve negative reinforcements (McLean, 2015; Waran *et al*., 2007). Many such interactions between human and horse, especially if use of negative reinforcements is inappropriately applied, may result in higher stress and fear levels (McLean and Christensen, 2017). Fearful animals are also more problematic to manage, as defensive reactions make working more difficult and can present a risk for human safety (Lansade *et al*., 2019).

Despite that, a lot of managing and training techniques are still based on the traditional methods, with a surplus of negative reinforcements, when a person often applies an unpleasant stimulus until the horse performs the desired behavior (Waran *et al*., 2007). In contrast, methods often defined as natural or sympathetic horsemanship consider the importance of positive human-horse interaction (Birke and Brandt, 2009) have slowly been developed. They allow humans to achieve success with the horse without using force. This is achieved through communication, mutual understanding and knowledge of the psychology, needs and natural behavior of horses (Birke, 2008; Waran *et al*., 2007). Compared to traditional methods, the natural methods have been shown to result in lower stress levels (Visser *et al*., 2009), more positive horse-human interaction during handling (Fureix *et al*., 2009) and lower reactivity (Polito *et al*., 2007).

One of methods based on natural horsemanship is Tellington-Jones equine awareness method -TTEAM that focuses on non-aversive approaches, consisting of different techniques of touch, movement and body language to establish safety and influence the behavior and welfare of animals (Curcio-Wolfe, 1996). TTEAM is specifically designed to help the horse to work or relax in a potentially stressful situation and allows a person to connect with a horse. For example, during handling or grooming, instead of using only a brush, TTEAM recommends touching the horse throughout the body with hands, to connect and assess if the horse is relaxed. Also, during halter-leading, the leash is in a relaxed position, with humans leading the way, but allowing the horse to move at its own pace (Tellington-Jones, 2006). The main scientific evidence of success with TTEAM are reduced stress levels and increased willingness to cooperate with humans in horses (Shanahan, 2003) and heifers (Ivemeyer *et al*., 2015).

In this study, we aimed to compare the effect of TTEAM and traditional method (T) of touch and leading on behavioral responses during handling. Based on the assumption that

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TTEAM leads to relaxation and increases safety, we hypothesized that TTEAM will improve the reception of contact and ease the handling of horses while being touched and led.

MATERIALS AND METHODS

Based on The National Animal Protection Act, no ethical approval was needed because the study did not involve laboratory animals and practices were non-invasive and non-experimental, therefore not considered as procedures on animals for scientific purposes.

Animals and housing

The study was conducted in May and June of 2019 at Lipica Stud Farm in Slovenia and included a herd of 22 Lipizzan mares. They were 4 years old and naïve to touch and leading. The mares were group-housed in a stable (20 m × 6 m) with deep straw bedding on the floor and with access to an outdoor area. The outdoor area (85 m × 45 m) was arranged outside the stable. In this area, animals were able to find the shade provided by the trees and water and hay were available ad libitum. The mares were given daily oats at 6:00 and at 7:00 they were let outside to graze. They returned to the stable at 18:00. For the purpose of this study, the management had to be changed slightly. The animals were given oats at 6:30 and the testing started at 8:00 and lasted until 11:00. After the testing, animals were released outdoors.

Procedure

The experimenter was a female who was unfamiliar to the mares at the onset of testing. Behavioral observations of animals were held inside (touch protocol) and outside (leading protocol) the home stable and lasted for 12 days, throughout four consecutive weeks. During the first two weeks, three consecutive observation days per week were used for application of T while during the following two weeks, three consecutive observation days per week for application of TTEAM. Since our mares were naïve to human contact and an initial application of TTEAM could have created a bond between a horse and human (Shanahan, 2003), applying TTEAM first could be reflected in horses perceiving T approaches less negative as they would without this prior positive experience.

Having a small number of horses available, we performed a priori power analysis using G*Power software that showed we did not reach the target sample size. For that reason, we designed a crossover study with each horse as its own control. Both handling methods were implemented on the same horses in the same order for each animal. Regardless of the method implemented, the protocol of behavioral responses was also carried out in the same order, starting with touching the mare (touch protocol) and continuing with leading the same mare on a leash (leading protocol) without breaks. Each mare was tested individually for approximately 10 min per day.

Touch protocol

In the touch protocol, mares were chained to the rack. For application of touch, brush was used in the T (Fig 1a). Upon entering the stable, the experimenter calmly announced her arrival and prior to physical contact with the brush, spoke to the mare in a calm and low voice. The experimenter held the brush in her right hand and moved on the mare's body, while holding the stopwatch in the left hand. The stopwatch was used to measure the time spent for the implementation of touch in three lines (Fig 1b), starting when the experimenter first touched the animal. The order of the brushing was from line A to B, to C.

In TTEAM, the experimenter also calmly announced the arrival at the stable and spoke calmly before the physical contact. Then, the experimenters' bare hand was used in the touch protocol instead of a brush. Before the implementation of the touch, the experimenter clasped the crest of the neck with the fingers of both hands and pressed (Fig 2a). After this initial phase, which lasted up to 1 min, the experimenter started with the touch phase using the right palm of her hand (Fig 2b). The palm and the fingers were stretched and relaxed (Fig 2c). In order to thoroughly assess the mares' reception of touch, the neutral parts (back, stomach; lines A-C) were contacted first, followed by sensitive body parts like ears which were not included during T (line D).

Once per testing day, the touch was assessed by observing the time spent for the implementation of touch and by rating responses to touch using a 6-point scale, modified after Janczarek *et al*. (2018) (Table 1).

Leading protocol

After completion of the touch protocol, mares were untied from the chain, attached to a leash and turned away from the rack. The experimenter stood parallel to the mare and motivated its movement by showing a moving forward gesture joined with a slight tension of the leash. The distance from the stable to the starting point by the tree was 20 m. The experimenter started the stopwatch and the animal was led to walk in steps from the starting point until it circled around a tree and continued walking on the right side back towards the starting point. The walking distance between the trees was 15 m.

During the implication of T, the mare was kept on a short leash (approximately 15 cm) to provide the best possible control, which restricted head movement. The direction of body movement was indicated with a strong pull on the leash. During leading, the experimenter was one step ahead of the mare. In instances a mare sped up, the experimenter reacted immediately by pulling down the leash and slowing down, or even turning the mare around its axle. If the mare was behind the experimenter or refused to continue walking, the experimenter gently tapped below the elbow or whipped on the thigh.

During the application of TTEAM, the experimenter led the mare on a long leash (length of approximately 100 cm).

This method allowed free movement of the head combined with a free pace choice. The mare was allowed to show a natural response to a situation (*e.g.*, standing alert, moving the head). If the mare stopped without any disturbances in the surrounding, TTouch Dingo approach described in Tellington-Jones (2006) was used.

At each testing day and during both methods, leading was assessed by observing the time spent walking on a leash and by rating responses to leading using a 6-point scale (Table 1). Scores for leading were based on the assessment of traceability to the experimenter and line walking, based after Janczarek *et al*. (2018) and Lansade *et al*. (2016) (Table 2).

Statistical analysis

Statistical analysis was performed using statistical software IBM SPSS Statistics for Windows. Means with standard deviations (SD) were calculated for descriptive statistics and graphs. Normality was assessed using the Shapiro-Wilk test, providing the assumption of normality was not violated. Data was analyzed using the generalized linear mixed model (GLMM) procedure. For all four models (touch scores, leading scores, touch duration and leading duration), the fixed effect of testing day (n = 12) and the random effect of individual mares (inter-individual variation; n = 22) was analyzed. For comparisons of multiple means (difference between individual testing days), a post-hoc Tukey HSD test with Bonferroni corrections was utilized and Eta Squared (η^2) was used to measure the effect size. Since we were also interested in more direct comparison in scores and durations between the methods, the differences were assessed using t-tests when comparing interval data and Mann-Whitney U tests when comparing ordinal data. To test the correlations, Spearman's correlation coefficient was used. Statistical significance was accepted if $P<0.05$ and tendency if $P<0.10$. The range of behavioral plasticity (intra-individual variation) was calculated using the coefficient of relative plasticity (CRP).

RESULTS AND DISCUSSION

By observing the behavioral responses of mares during the implementation of touch and leading, using two different

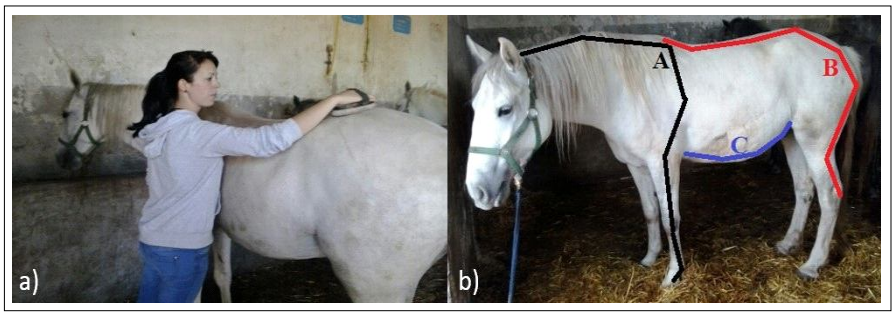
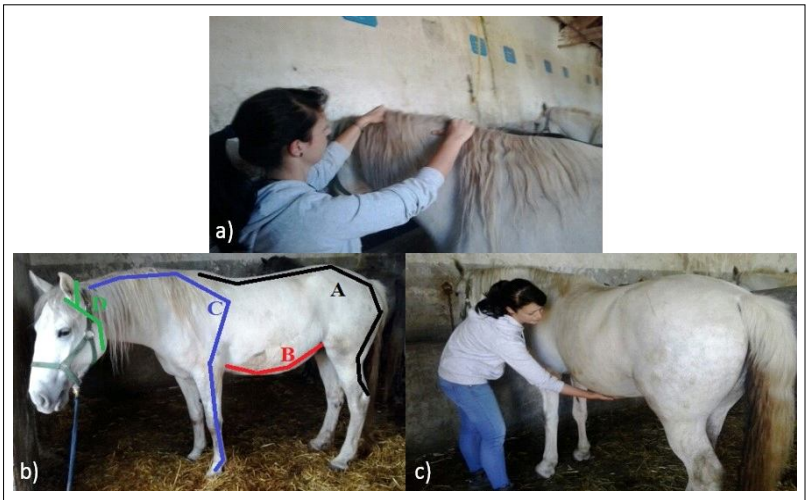


Fig 1: Implementation of T; a) Touch in B line; b) three lines of brushing: A- From poll over crest to pastern of front legs, B- From withers over back till hock, C- From the start till the end of barrel.



A) From withers over back till hock, B) From the start till the end of barrel, C) From poll over crest to pastern of front legs, D) From lower part of cheek to ear (touch ears).

Fig 2: Implementation of TTEAM; a) Relaxation phase, b) Lines of touching with a hand; c) Touch in B line.

handling methods, our findings show the improvement in mare's reception of touch and leading when using TTEAM compared to T. These results are in line with previous body of research showing the effect of TTEAM or similar natural methods on reduced fear, reactivity and physiological stress in young horses (Polito *et al*., 2007; Visser *et al*., 2009)

Touch protocol

A significant difference was found in the touch scores between mares ($F = 20.51$; $\eta^2 = 0.56$; $P<0.001$). The score ranged from 1.4 to 5 (Fig 3). Scores significantly differed between testing days ($F = 4.86$; $\eta^2 = 0.19$; $P<0.001$). During the first six days, the score was constant and there was no change in score when the method was changed. From the 8th to 12th day there was a gradual increase in scores (Fig 4). Regardless of the method, numerically the highest score (Fig 4) was found on the last day of the method application while the lowest score (Fig 4) on the 2nd day of the method implication. There was a significantly higher touch score in the TTEAM (4.6±0.9) compared to T (4.1±1.2; $Z = -4.45$; $P<0.001$; Fig 4). Generally, a wide spectrum of tactile interactions results in the activation of the central nervous system, by targeting neurocircuitry such as the orbitofrontal cortex and anterior cingulate cortex (Lindgren *et al*., 2012), which an individual usually perceives as pleasant (Morrison *et al*., 2010). In horses, proximity and tactile interaction, for example allogrooming is important for establishing and maintaining social bonds (Wolter *et al*., 2018). It translates also to horse-human interaction, as stroking or massage to some extent imitates natural allogrooming and has previously been reported to induce positive behavioral responses (McBride *et al*., 2004).

Touch also presents a social signal for safety in human-animal interaction, however, positive human-directed behavioral responses in horses, as a result of touching, have only been observed while touching preferred body parts for grooming, such as croup (McBride *et al*., 2004). Similarly, in our study, more positive behaviors towards the experimenter and better reception of touch resulting in higher touch scores, were shown during TTEAM. This may be the result of relaxation caused by the initial massage-like pressure to the neck (McBride *et al*., 2004). Also, the influencing factor may be the body part on which the touch was first applied. During TTEAM it was on the whither which is a preferred site for grooming, whereas during T it was around the ears, well known as a non-preferred site for grooming (McBride *et al*., 2004).

There was a significant difference in touch duration between mares ($F = 11.12$; $\eta^2 = 0.50$; $P<0.001$). The duration ranged between 10.9±2.6 s and 28.5±1.1 s (Fig 3). Duration significantly differed between testing days ($F = 8.67$, $\eta^2 = 0.29$; $P<0.0001$) but was consistent from 1st up to 6th day.

Table 1: Assessment protocols of touch and leading.

Score	Touch	Leading
0	Negative attitude towards experimenter, fear-related or agonistic behavior (*e.g*., walks backwards), does not allow to be touched.	Traceability: Very negative, Line: No Line
1	Somewhat negative attitude towards experimenter, it is not feasible to carry out one touch after three trials.	Traceability: Negative, Line: No Line
2	No negative attitude towards experimenter, calm, some signs of fear (*e.g*., occasionall retreat), needed two repetitions of one touch.	Traceability: Satisfied, Line: No Line
3	No negative attitude towards experimenter, calm, some signs of fear, one repetition of a touch.	Traceability: Good, Line: Bad
4	Positive attitude towards experimenter, calm, no signs of total relaxation (*e.g*., head tilts), accepts the touches.	Traceability: Good, Line: Bad
5	Positive attitude towards experimenter, calm, signs of total relaxation, accepts the touches.	Traceability: Excellent, Line: Excellent

Table 2: Description of traceability and line in the leading protocol.

Traceability	Definition
Very negative	Not complying with experimenter's commands, negative attitude towards experimenter (*e.g*., walks backwards).
Negative	Not complying with experimenter's commands, does not come from the stable.
Satisfied	Somewhat complying with experimenter's commands, some signs of stress (*e.g*., occasional retreat), steps out of the stable after a long persuasion (whip, Dingo).
Good	Somewhat complying with experimenter's commands, some signs of stress, some resistance at the exit from the stable.
Very good	Obeying experimenters' commands, some signs of stress.
Excellent	Immediately obeying experimenters' commands, relaxed.
Line	**Definition**
No line	Not walking in the line, rotations.
Bad line	Some deviating from the line.
Excellent line	Walking in the line.

The biggest difference in duration was between the 6th day and 7th day (P<0.001) and then significantly decreased on the 10th and 11th day. The touch duration was significantly higher in the TTEAM compared to T (t = -4.85, P<0.0001; Fig 4). In order to observe mare's reaction to touch of also less preferred grooming sites, an additional line for touching around the ears was added in TTEAM. Because of that, the results of touch duration are difficult to compare between the two methods and only duration within each method is worth discussion. During T the duration was consistent, whereas throughout TTEAM the duration decreased, the latter showing the mare's reception of touch was improved. This finding is further supported with higher touch scores (ρ = -0.55; P<0.001) being associated with decreased touch duration (ρ = -0.55; P<0.001).

Leading protocol

A significant difference was found in the leading scores between mares (F = 22.46; η^2 = 0.67; P<0.001). The scores ranged from 0 to 4.7 (Fig 3). Scores significantly differed between testing days (F = 10.74; η^2 = 0.34; P<0.001). During the first six days, the lowest score of 2.2±1.2 was recorded on the 1st day and the highest score of 4.5±1.5 on the 5th day. Up to the 5th day there was a gradual increase in the score. There was no change in score when the method was changed and up to 12th day the score was constant with no statistical differences. There was a significantly higher

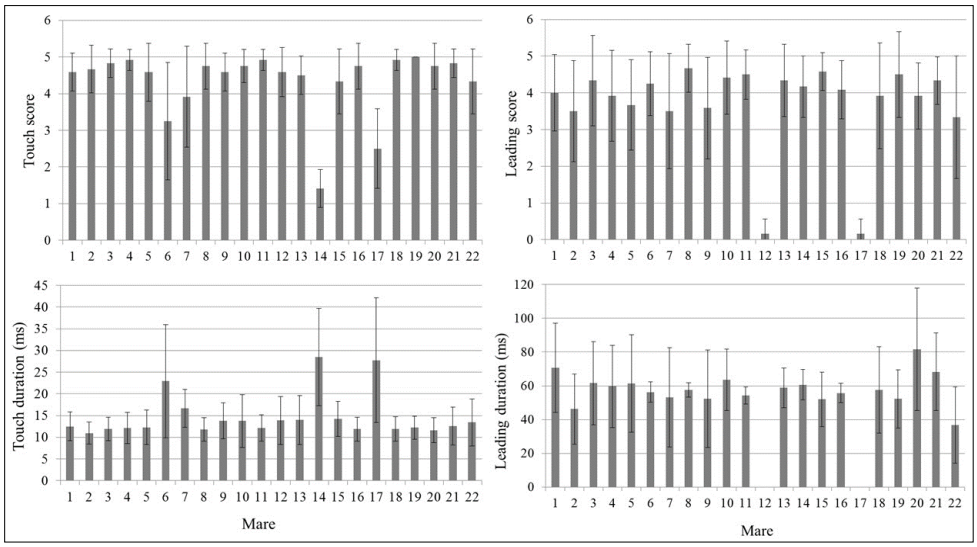
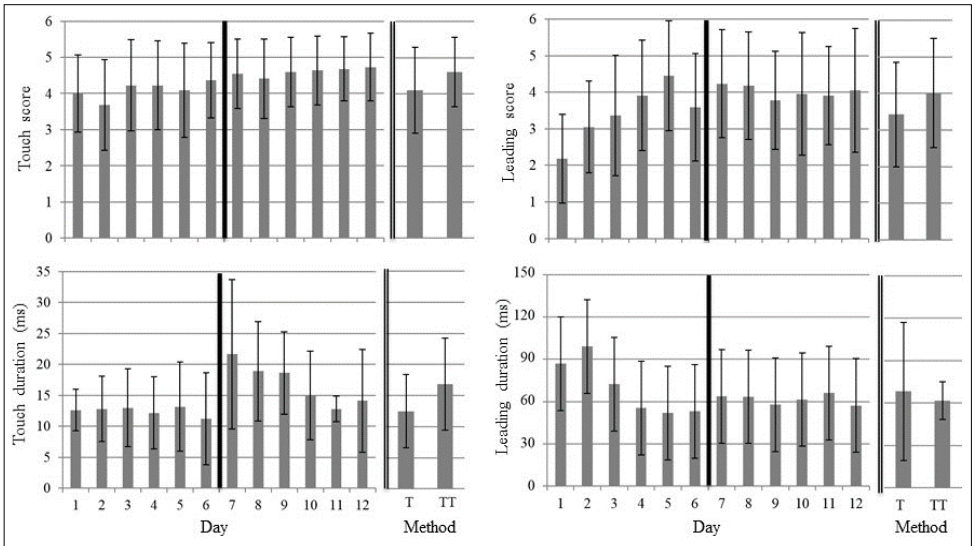


Fig 3: Scores and duration of touch (left) and leading (right) presented as means and standard deviations for each individual mare.



The bold line separates the two methods. The two lines separate data regarding testing days and methods.

Fig 4: Scores and duration of touch (left) and leading (right) presented as means and standard deviations for each testing day and method.

leading score in the TTEAM compared to T (Z = -3.47; P = 0.001; Fig 4). During leading, it appears that the absence of forced cooperation during TTEAM accounted for higher scoring compared to T. Positive reinforcements were proposed to induce positive attitude towards humans and better long-term horse-human interaction (Sankey *et al*., 2010). In contrast, the use of negative reinforcements may lead to stress and increased occurrence of agonistic behaviors, including aggression (McGreevy and McLean 2005). Considering correlations, higher leading scores were associated with lower leading duration (ρ = -0.33; P<0.001).

There was no significant difference in the leading duration between mares (F = 1.48, η^2 = 0.13; P = 0.10). Duration significantly differed between testing days (F = 3.43, η^2 = 0.16; P<0.0001). From 1st to 6th day the duration gradually decreased, with the longest time of leading being 98.9±94.4 s on the 2nd day and the lowest of 51.9±51.8 s (Fig 4) on the 5th day (P<0.001). Duration increased when the method was changed (P<0.001) but was then constant until the 12th day. There was no difference in duration between the methods (t = 1.57, P = 0.12; Fig 4). This is surprising knowing that during TTEAM mares were free to walk at their own pace and explore the environment and one would expect that it would be more time consuming. This found indifference, but higher leading scores and significant association between higher leading scores and decreased leading duration, TTEAM appears to be useful method for increasing horses' cooperation, reducing agonistic behaviors, while not being time consuming at the same time. The positive effects of TTEAM on leading were also reported by Shanahan (2003), who found training with TTEAM to help decrease loading time, saliva cortisol concentration and heart rate during trailer loading.

Although, on average, mares showed more positive behavior during TTEAM in the majority of the observed parameters, there were significant individual differences found within (intra-individual variability) and between mares (inter-individual variability). Intra-specific variation, calculated as CRP, ranged between 0.02 and 2.95. CRP differed between parameters and was 0.34±0.51 for touch score, the highest for leading score (0.92±0.61), 0.64±0.96 for touch duration and the lowest for leading duration (0.34±0.30). By finding intra-individual variability in behavioral plasticity, we highlighted the importance of horse's individuality, regardless of the handling method. This may not be surprising knowing that the experience of tactile interaction (Ahrendt *et al*., 2015) and response to handling (Hartmann *et al*., 2021) varies among individual horses. For this reason, not only handling method per se, but also other factors affecting handling responses should be taken into account when choosing the appropriate handling method for an individual horse.

For consideration in future research, we believe it is important to address certain limitations of our crossover study. Having a small sample size, we did not have enough power to generate scientifically valid and reproducible data if observing two separate groups of mares, each being tested with one handling method. Given the testing order, it could be argued that our mares could get habituated to humans, testing environments and procedures. However, it seems less likely this to happen, since habituation in our mares would translate into a decrease of negative behavioral responses, resulting in exponentially increased or constant scores. Such scoring was not observed in our study, implying the changes in behavior were not influenced by habituation to humans.

CONCLUSION

During TTEAM, the initial massage-like pressure on the neck and the use of bare hand seem to reduce the occurrence of aversive and stress-related behaviors during touch. Similar behaviors and better cooperation were also observed during leading, when free choice of pace, liberty to explore the environment and no forced cooperation were applied. Despite some limitations in the study design, the use of natural handling methods such as TTEAM may have a positive effect on the reception of touch and leading in Lipizzan mares but may vary depending on the individual behavioral plasticity of a horse. This highlights the importance of individuality when choosing an appropriate handling method for a particular horse.

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Conflict of interest

The authors declare no conflict of interest.

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