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Welfare assessment of horses ridden in the Costa Rica National Horse Parade

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Abstract. The National Horse Parade of Costa Rica (NHPCR) is an equestrian event held annually to celebrate the national day of the horse rider. Public concern regarding the welfare of horses and riders during the event has increased due to the removal of horses in poor body condition, spine injuries, and hoof problems. An Assessment of horse behavior and stress response during these events has never been conducted. The objective of this study was to evaluate the horse behavior, fecal glucocorticoid metabolites and body weight as indicators of welfare during the NHPCR. In the Experiment 1, body weight and fecal glucocorticoid metabolites were measured in 13 Costarricense de Paso horses participating in the NHPCR. All measures were recorded at the horses' home stable 48 hours before and 24 hours after the event. In Experiment 2, three stations (equally spaced along the 3-kilometer parade route) were used to randomly conduct a scan sampling of the behavior of 513 horses. Eye-wrinkle and eye-white presence were recorded on 48 horses using photographs taken during the event. Fecal glucocorticoid metabolites showed no significant differences ($p > 0.05$) at the two time points in the horses evaluated, and there was a significant weight loss (-7.08 kg) 24 hours after the event ($p = 0.00034$). The scan sampling provided evidence that traits such as sweating (60.74 %), bit chewing (54.70 %), and active gait (65 %) were more common than head tossing (28.88 %), neck above the withers (6.83 %), hyperflexion (28.71 %), and ears pinned backwards (31.19 %). A strong eye wrinkle was detected on 70 % of the horses, while only 16 % had the presence of eye-white. Although our results for fecal glucocorticoid metabolites were not conclusive, other behavioral traits indicated that over fifty per cent of the horses attending the NHPCR experience some level of stress.

Keywords: body weight, behavior, stress response, glucocorticoid metabolite

Evaluación del bienestar de caballos montados en el desfile nacional de caballos de Costa Rica

Resumen. El Tope Nacional de Costa Rica (NHPCR) es un evento ecuestre realizado anualmente para celebrar el día nacional del caballista. La preocupación pública sobre el bienestar de los caballos y jinetes ha aumentado debido a la cantidad de caballos sacados del evento debido a mala condición corporal, lesiones en columna, problemas de cascos y dificultades en carga y descarga. Aunque se han implementado acciones para salvaguardar el bienestar de los caballos en años recientes, no se ha evaluado su comportamiento y los niveles de estrés durante este evento. El objetivo del estudio fue evaluar el comportamiento, los metabolitos de glucocorticoides en heces y el peso corporal como indicadores de bienestar de caballos durante el NHPCR. En el Experimento 1 se determinó el peso corporal y los niveles de cortisol fecal en 13 caballos Costarricense de Paso 48 horas antes y 24 horas después del evento. En el Experimento 2, tres estaciones (equidistantes a lo largo de la ruta de 3 kilómetros del recorrido) se usaron para evaluar el comportamiento de 513 caballos. Se registró la presencia de arrugas y conjuntiva del ojo en 48 caballos con fotografías tomadas en el evento. No hubo diferencias significativas ($p > 0.05$) en el análisis de metabolitos de glucocorticoides fecales en los dos momentos de muestreo y hubo una pérdida significativa ($p = 0.00034$) de peso 24 horas después del evento (-7.08 kg). Se halló evidencia de que características como la sudoración (60.74 %), mordisqueo del bocado (54.70 %) y paso activo (65 %) fueron más comunes que la sacudida de cabeza (28.88 %), cuello levantado (6.83 %), hiperflexión del cuello (28.71 %) y orejas posicionadas hacia atrás (31.19 %) durante el evento. Se detectó una arruga fuerte en los ojos (70 %) y presencia de la conjuntiva (16 %) en los caballos. Aunque los resultados de glucocorticoides fecales no fueron concluyentes, otras características de comportamiento indicaron que más de la mitad de los caballos asistentes al NHPCR experimentan algún nivel de estrés.

Palabras clave: peso corporal, comportamiento, respuesta de estrés, metabolitos de glucocorticoides

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Avaliação do bem-estar de cavalos montados no desfile nacional de cavalos da Costa Rica

Resumo. O Tope Nacional de Costa Rica (NHPCR) é um evento equestre realizado anualmente para celebrar o Dia Nacional do Cavaleiro. A preocupação do público com o bem-estar dos cavalos e cavaleiros aumentou devido ao número de cavalos retirados do evento com inadequadas condições corporais, lesões na coluna vertebral, problemas nos cascos e dificuldades de carga e descarga. Embora ações tenham sido implementadas para salvaguardar o bem-estar dos cavalos nos últimos anos, seu comportamento e os níveis de estresse durante este evento não foram avaliados. O objetivo do estudo foi avaliar ocomportamento, os metabolitos de glicocorticoides em fezes e o peso corporal como indicadores de bem-estar dos equinos durante o NHPCR. No Experimento 1, o peso corporal e os níveis de cortisol fecal foram medidos em 13 cavalos da Costa Rica Paso 48 horas antes e 24 horas após o evento. No Experimento 2, três estações (igualmente espaçadas ao longo do percurso de 3 quilômetros) foram usadas para avaliar o comportamento de 513 cavalos. A presença de rugas e conjuntiva ocular foi registrada em 48 cavalos com fotografias tiradas no evento. Não houve diferenças significativas ($p > 0.05$) na análise dos metabólitos fecais de glicocorticoides nos dois momentos de amostragem e houve perda de peso significativa ($p = 0.00034$) 24 horas após o evento (-7.08 kg). Evidenciou-se que características como sudorese (60.74 %), mordeduras (54.70 %) e marcha ativa (65 %) foram mais comuns do que sacudir a cabeça (28.88 %), pescoço levantado (6.83 %), hiperflexão do pescoço (28.71). %) e orelhas posicionadas para trás (31.19 %) durante o evento. Forte enrugamento dos olhos (70 %) e presença da conjuntiva (16 %) foram detectados nos cavalos. Embora os resultados de glicocorticoides fecais tenham sido inconclusivos, outras características comportamentais indicaram que mais da metade dos cavalos atendidos no NHPCR experimentam algum nível de estresse.

Palavras-chave: peso corporal, comportamento, resposta ao estresse, metabolitos de glicocorticoides

Introduction

The National Horse Parade of Costa Rica (NHPCR) is a cultural event celebrated on December 26 to celebrate the National Horseman's Day. Around 2000 horses participate in this event every year, being ridden in a three-kilometre route in the streets of San José, Costa Rica. In recent years, incidents have raised welfare concerns for equines participating in the event. According to Camacho (2017), the National Service for Animal Health (SENASA) had to remove sixteen horses from the event in 2017 due to various traumas. Likewise, five sets of non-permitted spurs carried by the riders were seized. Even though SENASA and the Municipality of San José enforce local regulations to safeguard the welfare of participating animals, the question of whether horses undergo stress during the event has not been addressed empirically (Pena, 2015).
Physiological measures provide insight into how animals perceive environmental challenges (Wikelski and Cooke 2006). The two main stress axes involved in triggering the release of stress hormones are the autonomic nervous system and the hypothalamic-pituitary-adrenocortical (HPA) axes (Flauger 2010). The neuroendocrine stress response is a cascade of events mediated by the HPA axis. When an organism encounters a stressor, the HPAaxis is activated to initiate an appropriate centralized stress response (Möstl *et al*. 2002). The end products of the HPA axis are glucocorticoids (cortisol and corticosterone), steroid hormones, that play a key role in mobilizing glucose, mediating energy balance and regulating animal metabolism, nutrition, reproduction, and immunity (Cox *et al.* 2010). Thus, glucocorticoid concentrations are not equivalent to measuring a stress response directly, rather, they represent an end product from a signaling cascade (MacDougall Shackleton *et al.* 2019).

Glucocorticoid metabolites can be used as indicators of stress in horses. Kruguer (2010) found a positive correlation between fecal glucocorticoid metabolites and serum cortisol in horses. In addition, fecal glucocorticoid metabolites have been used in other equine species, such as zebras. Franceschini *et. al* (2008) reported that fecal glucocorticoid metabolites have been helpful in measuring stress caused by transportation, management, and acclimation to captivity in zebras. The same authors reported that fecal glucocorticoid metabolites were higher during the first days of captivity and then decreased when the animals became accustomed to the unfamiliar environment.

The behavior assessment is also a reliable indicator of stress in horses. The position of the neck, ears and nostrils have been used as indicators of welfare (Hintze *et al.* 2016). According to Hintze *et al.* (2016), eye wrinkles and eye expression could be useful visual indicators that may allow the identification of stress in

horses. Moderate to high stress levels have been reported to increase oral behaviors, the presence of flared nostrils, and flattened or pinned ears, which has been correlated with an increase in heart rate and salivary cortisol (Young *et al.* 2012). Therefore, learning how to recognize eye wrinkles and behavioral indicators of stress during horse events could be a valuable tool for horse owners and entities responsible for regulating animal welfare.

Given the incidents presented in previous years related to horses' welfare during the NHPCR, additional research can provide insight into the level of stress the animals experience as well as identify strategies to amend such problems. Therefore, the objective of this study was to evaluate the horse behavior, fecal glucocorticoid metabolites and body weight as indicators of welfare during the NHPCR.

Materials and Methods

Ethics Approval, local and animals

This study was performed in San José, Costa Rica, from November 2019 through August 2020. Thirteen Costarricense de Paso breed horses (3-12 years) were randomly selected from a single farm where horses were kept under similar conditions of housing (3.5 x 3.5 m stalls), feeding (forage and concentrate twice per day), and exercise (once per week). All the horses had previously attended the NHPCR.

Experiment 1: Body weight and fecal glucocorticoid metabolites measurements

Both horses' body weight and fecal samples were taken at two-time points at their individual stables (48 h before and 24 h following the event) as recommended by Palme *et al*. (1996) and Möstl *et al.* (1999). Horses were weighed by using a heavy-duty scale that was placed on a pliable wooden platform. Fresh fecal samples were hand-collected from the stalls and pieces of grass, hay, feed, or litter were carefully removed from the sample using a brush to avoid contamination from other materials in the analyses. Feces were mixed and homogenized by reducing their particle size by hand in individual aluminum containers and 100 grams of each sample were weighed and placed in individual plastic bags for later storage at -20 °C in a freezer in the Anatomy and Physiology Laboratory of the Department of Animal Sciences of the University of Costa Rica.

Glucocorticoid metabolite analysis was performed using a commercially available 11-oxoetiocholanonolone ELISA kit (Cayman chemical ®). The analytical sensitivity of the assay was 0.2 ng/ml and the intra-essay coefficient was 11.4 %, following the methodology suggested by Flauger *et al*. (2010).

Experiment 2: Observational study

An observational study was performed during the NHPCR of 2019 across three stations equally spaced (approximately 500 m) along the 3-kilometer parade route (start, the halfway point, and end of the event). Three trained observers per station performed scan sampling using an ethogram (Table 1 and Table 2) on both horses and their respective riders for a total of 513 observations. Observers were positioned approximately 2 meters away from the analyzed horses and observations were recorded every 3 minutes (and lasted 3 minutes) on a random horse. Observations were taken from 12p.m. to 5p.m., which corresponds to the duration of the event.

Table 1. Ethogram utilized for conducting horse behavior measures during the National Horse Parade of Costa Rica.

Variable	Status	Ethogram interpretation
Sweating	Present	Visible and evident humidity (wet coat) was present under the saddle and /or the neck and flanks.
	Absent	Nonvisible or evident humidity was present under the saddle and/or the neck and flanks.
Bit chewing	Present	Actively chewing the bit and moving the lips side to side at least once during the 3 minutes of observation.
	Absent	Non-visible active chewing of the bit or moving the lips side to side during the 3 minutes of observation.
Head tossing	Present	Head tossed up and down or sided to side at least once during the 3 minutes of observation.
	Absent	Non-visible head tossing during the 3 minutes of observation.
Active gait	Present	The horse maintains an active gait (prancing) in the same spot or while in movement during the 3 minutes of observation.
	Absent	No presence of active gait.
Ear position	Normal	Ears at any other position except pinned backwards during the 3 minutes of observation.
	Pinned backwards	Ears pinned very close to its head at least once during the 3 minutes of observation.
Neck position	Normal	The horse maintains a balanced position on the neck compared to the withers.
	Upwards	The horse keeps the neck at an angle greater than 45° from the withers.
	Hyperflexion	The horse's mouth is as close to the chest during the 3 minutes of observation.

Table 2. Ethogram utilized for conducting rider behavior measures during the 2019 NHPCR.

Variable	Status	Ethogram interpretation
Excessive kicking	Present	The rider is excessively kicking on the flanks of the horse during the 3 minutes of observation
	Absent	No evident excessive kicking on the flanks of the horse during the 3 minutes of observation
Excessive use of the whip	Present	The rider is actively hitting the horse with the whip during the 3 minutes of observation
	Absent	The rider may have a whip, but it is not actively hitting the horse with it during the 3 minutes of observation.
Aggressive behavior	Present	The rider is noisy (yelling) or bothering other horses and/or riders during the 3 minutes of observation.
	Absent	Calm rider
Presence of alcohol	Present	Alcoholic beverages were visible in the rider's hands or in the horse's saddle. Rider was drinking during the 3 minutes of observation.
	Absent	Alcoholic beverages were not visible in the rider's hands or in the horse's saddle. Rider iwa not drinking during the 3 minutes of observation.

Eye wrinkles and eye-white assessment

The visual assessments for eye wrinkles and eye-white were taken at the second station through a photographic record by using a semi-professional digital camera (Panasonic Lumix DMC-FZ70). Pictures of 50 random horses were taken during the event every 10 minutes to collect data from different sections and time points throughout the day. After the event, the photographic record went through a screening process (pictures with low resolution) to choose the best pictures to be analyzed, following the methodology of Hintze *et al* (2016). The eye wrinkles were assessed and classified into three categories (no wrinkle, weak or strong), while eye-white was assessed by presence or absence.

Statistical analysis

Experiment 1

Data were analyzed with R Studio software R v3.6.1 (R Core Team, Vienna, Austria). The experimental design consisted of a one-factor analysis corresponding to the time points when data were collected (pre- and post-event). A paired t-test was conducted to evaluate changes in both fecal glucocorticoid metabolites and body weight of the horses pre and post event.

Experiment 2

Data for the observational variables were analyzed by a Pearson Chi Squared test in R Studio software. The variables were treated differently for each observation station, allowing them to estimate the probabilities of the behavior occurring at each station. Descriptive statistics were used to describe the eye wrinkles and eye-white present in the horses participating in the NHPCR.

Results and Discussion

Experiment 1: Body weight and fecal glucocorticoid metabolites

The feces collected in this experiment had similar ($p > 0.05$) concentrations of glucocorticoid metabolites at the two time points for each horse. Body weight of horses was significantly ($p < 0.05$) lower after the event (Table 3).

Table 3. Fecal glucocorticoid metabolites (FGM) and body weight of thirteen horses attending the National Horse Parade of Costa Rica.

Variable (units)	Timepoint	Mean	Standard deviation	p-value
FGM	Before	0.80 $ng.ml^{-1}$ ($8.0\ ng.g^{-1}$)	0.29	0.1889
	After	1.02 $ng.m^{l-1}$ ($10.2\ ng.g^{-1}$)	0.29	
Body weight	Before	451 Kg	53.2	0.0013
	After	446 Kg	49.8	

Previous studies have found that animals learn to cope with situations expected to generate stress by manifesting different behaviors (Fureix *et al*. 2013). Stressful situations can trigger the release of glucocorticoid metabolites, remaining at levels higher than normal during events such as the NHPCR. Merl *et al*. (2000) measured fecal glucocorticoid metabolites in stallions following castration, known to be a factor that causes stress in equines. Before castration, these authors report median concentrations of 10.5 nmol/kg feces (3.19 ng/g^{-1}) and after castration values increased to 26.2 (7.97 ng/g^{-1}) and 50 nmol/kg feces (15.22 ng/g^{-1}). In addition, Hinchcliffe *et al*. 2021 found that fecal glucocorticoid metabolites were elevated from the second day following a stressor (a roundup) and then slowly returned to basal levels over the next 2 weeks. Franceschini *et al*. (2007) monitored fecal glucocorticoid metabolites in zebras from the time of capture, during captivity and post-release as an indicator of the stress of translocation and acclimation to a new environment. These authors found that when held in pens at Meru Park 3–4 and 5–6 weeks after capture, the zebra had higher fecal glucocorticoid metabolites than either at capture or compared to non-translocated controls.

Despite the similar concentrations of fecal glucocorticoid metabolites found in this study pre and post event, horses showed behavioral indicators of stress, which are also considered stressors that could activate the HPA axis during the NHPCR. Pawluski *et al*. (2017) compared horses' baseline levels of cortisol and several welfare indicators and revealed that horses whose welfare was clearly compromised (as indicated by unusual ears backward position, presence of vertebral problems or hematological anomalies, e.g., anaemia) also had lower levels of cortisol (and metabolites). The results of our study expand earlier findings evidencing low or no change in cortisol levels after intensive training or racing of Thoroughbred fillies (Nogueira *et al*. 1997) or chronic sub-optimal living conditions of horses (Visser *et al*. 2008). This could be evidence that the HPA axis could have an attenuated activity when welfare is compromised.

Low levels of glucocorticoids and/or decreased HPA reactivity after different types of chronic stressors have been reported in several species, including farm animals and humans (Herman *et al*. 2016). The results of our study can be explained by the "general adaptation syndrome", a three-stage process that describes the physiological changes the body goes through when under stress (Selye 1956). The third phase of the "general adaptation syndrome" is exhaustion, where the organism is no longer able to cope with stress, thus making stress hormones not to be released as normal (Selye 1956).

Another factor that could explain our results is the "allostatic overload" mechanism in the case of chronic stress and the development of pathologies in humans (McEwen *et al*. 2003), which may be extended to other species, such as horses. According to this mechanism, stress hormones play a key role in maintaining homeostasis through change (allostasis). When allostasis mediators, such as cortisol, are released in response to stressors or lifestyle factors (diet, sleep-wake cycles and exercise), they facilitate adaptation and are generally beneficial. However, when the stressor is sufficiently intense or prolonged (chronic), then the allostatic load can increase dramatically until it reaches "allostatic overload". Allostatic overload can then lead to wear and tear of the system on the body and brain such that it no longer responds appropriately to the change in allostasis (McEwen *et al*. 2003). It might be possible that chronic confrontations with repeated welfare-related stressors in horses involving an allostatic overload, may be reflected by maintaining the levels of fecal glucocorticoid metabolites when exposed to an event such as the NHPCR. The horses evaluated had restricted access to social contact and roughage (except hay and alfalfa) as well as intensive riding techniques (rein tension, high hands or amplified aids, strong control attempts on horses' movements) were used in the stables, which can alter welfare and animal's perception of its environment, creating an attenuated HPA axis as explained previously.

The lack of difference in fecal glucocorticoid metabolites could also be attributed to an adequate management during the event (i.e., careful transportation and acclimation before and during the event), which might have reduced stress and maintained the comfort of the horses without altering the concentration of fecal glucocorticoid metabolites. Also, the horses that participated in this study have been previously exposed to or participated in events like the NHPCR, which can help to decrease the likelihood of having stressed animals. Stull and Rodiek (2000) indicate that a decrease in body weight could occur due to dehydration during transport or exercise. The horses used in this study spent at least 12 hours since they left the stables for the NHPCR and until they returned later that same day, limiting the forage and concentrate intake for half a day.

Harris (2015) reports that the energetic response to acute stress is determined by the nature and severity of the stressor. Typical responses to acute stressors

include inhibition of food intake, increased heat production, and increased activity with sustained changes in body weight, behavior, and HPA reactivity. These responses were common in horses attending to the NHPCR, which can explain body weight loss found after the event.

Experiment 2: Observational study

The behavior indicators evaluated were significantly different ($p < 0.05$) between the stations (Table 4). Except for head tossing and active gait, all the behaviors consistently showed higher probabilities of occurring in the second and third stations. The high probabilities (> 0.50) found for sweating, bit chewing and ear position in the second and third stations, indicate that horses may be experiencing stress and exhaustion as they progress in the NHPCR.

Table 4. Behavioral recordings on horses participating in the National Horse Parade of Costa Rica.

Condition	Absence (%)	Presence (%)	Chi Square p-value	Station 1 probability	Station 2 probability	Station 3 probability
Sweating	39.3	60.7	< 0.01	0.345	0.712	0.655
Bit chewing	45.3	54.7	0.044	0.477	0.592	0.587
Head tossing	71.1	28.9	0.032	0.347	0.288	0.221
Active Gait	65.1	34.9	0.013	0.376	0.256	0.399
Ear position	31.2	68.8	< 0.01	0.637	0.655	0.784

* Probabilities are shown only for variables with a significant p-value.
* Probabilities correspond to the chances of a horse showing a specific behavior at each of the stations.

Almost one third ($p < 0.05$) of the horses visually assessed showed a hyperflexion of the neck during the NHPCR (Table 5). Several studies have suggested that hyperflexion may negatively affect the horse and compromise welfare, causing impaired vision, airway obstruction, overstretched muscles and ligaments and delayed neuromuscular transmission (Von Borstel *et al*. 2009, Cehak *et al*. 2010, Elgersma *et al*. 2010). Christensen *et al*. (2014) mentioned that not only hyperflexion of the neck is uncomfortable and painful for the horse while riding, but it is also a concern in terms of animal welfare.

Table 5. Neck position on horses attending the National Horse Parade of Costa Rica.

Condition	Normal (%)	Upwards (%)	Hyperflexion (%)	Chi Square p- value
Neck Position	64.5	6.8	28.7	< 0.01

Alcohol consumption by the riders participating in the NHPCR represented a small proportion in this study (Table 6), indicating that strict enforcement was applied during the event. The rider's behavior may result in stress, exhaustion, or pain in the horses. Overall, rider behavior was mostly nonaggressive, with low use of the whip or kicking, which could indicate that this factor did not trigger stress in the horses attending NHPCR.

Table 6. Rider behavior evaluated during the National Horse Parade of Costa Rica.

Condition	Absence (%)	Presence (%)	Chi Square p-value	Station 1 probability	Station 2 probability	Station 3 probability
Alcohol consumption	91.4	8.6	< 0.01	0.1077	0.0196	0.1212
Excessive kicking	97.3	2.7	0.6243	-	-	-
Excessive whip use	96.3	3.7	0.8946	-	-	-
Aggressive rider behavior	97.1	2.9	0.2926	-	-	-

* Probabilities are only shown for variables with a significant p-value.
* Probabilities correspond to the chances of a horse showing a specific behavior at each of the stations.

Visual assessment of eye-white and eye-wrinkles showed that roughly two-thirds of the horses evaluated had strong eye wrinkles (Table 7). In contrast, a small percentage (16.7 %) had a visible eye-white presence.

Table 7. Eye wrinkle condition in horses attending the National Horse Parade of Costa Rica.

Wrinkle condition	Percentage (%) n = 48
No wrinkle	12.5
Weak	16.7
Strong	70.8

Eye wrinkles (also known as worry wrinkles) have been empirically associated with stress and pain among equine owners, professionals, and veterinarians (Merkies *et al.* 2019). A negative emotional state tends to be associated with wrinkles formed above the eyeball (Hintze *et al*. 2016). The Equine Facial Action Coding system describes this expression as the inner brow raiser (levator angulu oculi medialis muscle and corrugator supercili muscle), which increases the perceived size of the eye, but not the aperture of the eye (Wathan *et al*. 2015).

Hintze *et. al* (2016) conducted a study using different tests to provoke a positive or negative stimulus on the horse and found that eye wrinkles and eye-white can be useful indicators of fear or pain. They evaluated qualitative impression (first subjective impression of the expression of eye-wrinkles), eyelid shape, markedness of the wrinkles, presence of eye-white, number of wrinkles, and the angle between the line through the eyeball and the highest wrinkle. In our study, we followed Hintze *et al.* (2016) methodology but only the presence of eye-wrinkles and eye-white were evaluated. Within the randomized pictures taken during the event, we found that most horses had a strong eye wrinkle (70 %), and a smaller percentage had visible eye-white (16.7 %), indicating that the NHPCR could be generating stress in the animals (Table 7).

Conclusions

Despite the lack of difference in fecal glucocorticoid metabolites found in this study, indicators such as the presence of eye wrinkles and eye-white indicate that a moderate proportion of the horses attending the NHPCR experience some level of stress. In addition, the loss in body weight of the horses from experiment 1 can be mainly attributed to dehydration due to the number of hours spent in the NHPCR, which creates a different environment for the horse, affecting their feeding and drinking schedule and intake. Implementing "rehydration stations" (utilizing disposable recipients for each horse to avoid sanitary issues) during the event can attenuate the effects of this issue, allowing horses to drink small doses of water throughout the event. Horse owners must be aware that previous conditioning (by progressive and consistent training) and offering hay during transportation may be factors that could positively impact animal welfare during the NHPCR. The presence of active sweating, bit chewing, and ears pinned backwards found in the observational study revealed that a higher percentage of the horses might be presenting stress responses during the NHPCR. The higher probabilities found for sweating, bit chewing, active gait and, ears pinned backwards at stations 2 and 3 can be attributed to exhaustion. A closer follow-up of each horse at different moments through the NHPCR could provide further information on the potential of fecal glucocorticoid metabolites to assess stress in horses. Other variables, such as heart rate variability or eye temperature, could support our findings of whether the NHPCR is causing stress to the horses participating. With a larger scale welfare assessment, the organizing authorities could identify stress in the overall event. Eye-white and eye-wrinkles are rapid and low-cost indicators that may be evaluated during the NHPCR, allowing the organizing authorities to assess stress in horses during the event. Specific training about animal welfare before the event would allow the organizing authorities of the NHPCR to reduce the percentage of horses presenting welfare issues or discomfort and promptly address the issue or remove the animal from the event.

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