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IS HEART RATE A RELIABLE INDICATOR OF ACUTE STRESS IN HORSES *(EQUUS CABALLUS)*?

ESTE FRECVENȚA CARDIACĂ UN BUN INDICATOR DE STRESS ACUT LA CAI (*EQUUS CABALLUS*) ?

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ABSTRACT

Acute stress triggers in equines a fight or flight response, thus increasing the probability of injury for both human handlers and horses themselves. Nonetheless, this encourages the use of training and handling methods that drastically affect the welfare of horses. This study investigates the behavioural and physiological response of equine subjects to acute stress, aiming to assess whether heart rate alone could be a reliable stress indicator. Forty-nine healthy mares were exposed to acute stress by temporarily separation from their foals. Heart rate was recorded using a Polar H10 heart rate sensor before and after foal was removed from his dam. Behavioural stress indicators such as head position, ear movement, front leg pawing, nostrils dilatation, vocalisation and overall posture were used to confirm acute stress. Results indicated an increase in both heart rate and ethogram score as well as a positive correlation (p=0.018) between these parameters during the acute stress episode, confirming the reliability of heart rate to predict acute stress in horse subjects. The findings highlight the reliability of heart rate to predict acute stress in horses as an accessible tool to be used by non-professionals. This research contributes to the development of more effective welfare practices, and it opens the direction to further research exploring accessible, non-invasive techniques to detect stress in horses.

Keywords: acute stress, equine, ethogram, heart rate, welfare

REZUMAT

Stresul acut declanșează la cabaline un răspuns de atac sau fugă, crescând astfel probabilitatea de accidentare atât a manipulatorilor umani, cât și a cailor. Acest lucru încurajează utilizarea metodelor de antrenament și manipulare care afectează drastic bunăstarea cailor. Acest studiu investighează răspunsul comportamental și fiziologic al cailor supuși stresului acut, cu scopul de a evalua dacă doar ritmul cardiac ar putea fi un indicator de stres de încredere. Patruzeci și nouă de iepe sănătoase au fost expuse la stres acut prin separarea temporară de mânji. Frecvența cardiacă a fost înregistrată folosind un senzor de ritm cardiac Polar H10 înainte și după ce mânzul a fost îndepărtat de iapa mamă. Indicatorii comportamentali, cum ar fi: poziția capului, mișcarea urechilor, bătaia cu piciorul anterior, dilatarea nărilor, vocalizarea și postura generală au fost utilizați pentru a confirma stresul acut. Rezultatele au indicat o creștere atât a ritmului cardiac, cât și a scorului etogramei, precum și o corelație pozitivă (p=0,018) între acești parametri în timpul episodului de stres acut, confirmând fiabilitatea ritmului cardiac de a prezice stresul acut la cai. Descoperirile evidențiază fiabilitatea ritmului cardiac pentru a prezice stresul acut la cai ca un instrument accesibil pentru a fi utilizat de către non-profesioniști. Această cercetare contribuie la dezvoltarea unor practici de bunăstare eficiente și deschide direcția către cercetări ulterioare care explorează tehnici accesibile, non-invazive pentru a detecta stresul la cai.

Cuvinte cheie: stres acut, ecvine, etogramă, ritm cardiac, bunăstare

Equine stress is expressed by the triad hormonal, neural. and behavioural responses (13). The understanding of the physiology of stress is crucial for improving their welfare (14). Stress is a complex process regulated by mainly two body systems: the hypothalamic-pituitary-adrenal axis (HPA axis), with cortisol as the main stress biomarker, and the autonomic nervous system (19). Stress activates the HPA axis, starting with the hypothalamus releasing corticotrophin-releasing hormone (CRH) that stimulates the pituitary gland to secrete adrenocorticotropic hormone (ACT H), which then signals the adrenal glands to release cortisol (16). The sympatho-adrenal-medullary (SAM) axis is activated almost instantaneously by stress, leading to the release of catecholamines (adrenaline and noradrenaline) from the adrenal medulla (12). These hormones prepare the body for a 'fight or flight' response by increasing heart rate, blood pressure, and blood flow to muscles. Stress typically causes an increase in heart rate and a decrease in heart rate variability, which can be measured to assess stress levels (23, 26). In the last decades, there has been a noticeable increase in the interest in improving equine welfare. Equine welfare encompasses the physical and psychological well-being of horses with a focus on positive and negative emotions (3, 18) thus encouraging the use of non-invasive methods.

Stress can be assessed using non-contact and non-

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invasive methods such as the ethogram or HRV. (9). Decoding the facial body language, i.e., the ethogram, is a method used in traditional horsemanship as well as in scientific research. (2, 17). Recent studies show that the assessment of heart rate and especially heart rate variability can give reliable information about nervous excitability (7). Illness, pain, and physical discomfort are some of the causes that can influence the emotional well-being of horses. In addition to these, the environment that provides the sensory inputs, and the interactions with conspecifics and with people are essential factors that regulate the emotional status of equids (21). Detecting acute stress, and emotions such as fear, panic, or rage are essential not only to ensure the well-being of the species but also the positive human-horse interaction for both subjects involved (8). Assessing the valence and intensity of emotions can lead to successful communication and training based on positive reinforcement. Understanding emotional arousal can avoid accidents and smoothen the relationship with horses, even for beginners or those working with unfamiliar horses (6). Horses' interaction with humans is still a source of distress for humans and horses as well. Fight or flight responses occur in milliseconds and can cause accidents harming either of the subjects (1). In human athletes, fitness trackers are widely used to assess their fitness level, the ability of the body to adapt to certain situations, as well as several physiological and even pathological parameters, e.g., arrhythmias (25). Fitness tracker devices have been largely used to monitor horses in training and in different physiologic situations. (5, 24). However, the validity of some of these devices has not yet been assessed (23). Polar h10 heart rate monitors have been widely used in endurance equestrian sports in recent years, becoming very effective devices in evaluating horses' heart rate instantly and at a distance (20). The aim of this study was to evaluate the validity of heart rate measurements assessed with PolarH10 monitors to detect acute stress in equine subjects. Our hypothesis was that heart rate could indicate acute stress in horses and that the accessibility and simplicity of the technique might increase the welfare of horses and the understanding of horses' behaviour by non-professionals.

MATERIALS AND METHODS

Animals

The study involved the recording of the data from 49 different mares, with an average age of 12 (M = 11.69, SD = 4.38). Of the 49 horses, the largest percentage of them were (n=15) mixed breed, with CSR (n=10), Arab (n=8) and Lipizzaner (n=6) making up larger percentages of the sample. All mares had foals aged 2 to 6 months old at side.

Mares were housed around the Transylvania region in Romania in stalls of 12 to 16 square meters with straw bedding and automatic waterers.Mares received ad libitum hay and a muesli supplementation for lactating mares (Cavalor, Probreed Mix) two times per day. Mares and foals were turned out a minimum of 3 hours per day in grassy paddock areas.

Experimental design

In the first stage of the experiment, every mare was fitted with a heart rate sensor (Polar H10) attached to an elastic chest strap (Polar®). A video camera (GoPro Hero10®) was placed on a corner of the stall, making it possible to supervise the entire area. After these procedures, the mare was left alone for 15 minutes to accommodate the chest strap (Fig. 1).

Resting basal heart rate was recorded after 15 mi-

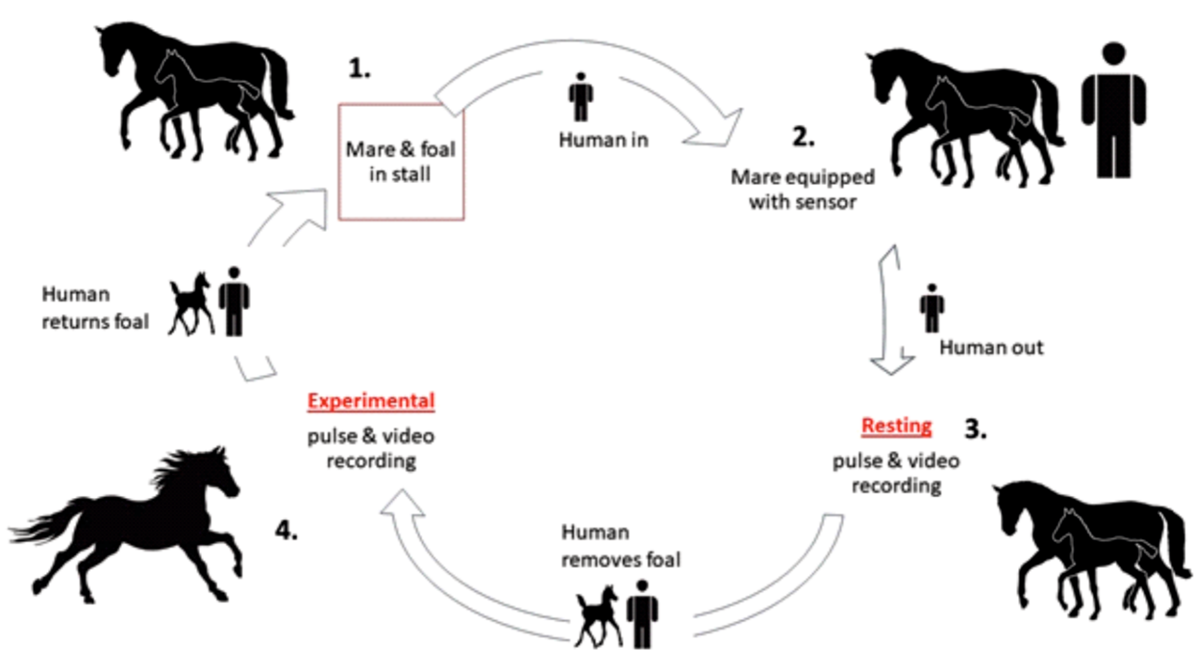

Fig. 1. Diagram of the study design showing the main stages of the experiment. In red data recording instances

nutes via the Polar Equine mobile app, concomitantly with the video recording of the behaviour for a period of 2 minutes. Next, a trained operator gently removes the foal from the stall and out of the sight of the mare for a period of 2 minutes. Experimental heart rate and behaviour are recorded continuously for the entire period. After 2 minutes, the foal is returned to the mare, and recordings stop (Fig. 1).

Measurements

Heart rate

Heart rate was recorded first as **resting** with the mares calm in their habitual environment, followed by **experimental** measurements after stress was induced (foal removed). Heart rate measurements were obtained using a H10 Polar sensor placed on the left area of the chest, under the midline. The sensor was attached to an elastic chest strap (Polar) that was previously sunk in water. Lubricating gel was applied to the rubber part of the chest strap in order to ensure better conductivity. Isopropyl alcohol was used whenever artefacts were observed on EKG. Data was recorded via the Polar Equine phone app on a phone that was placed just outside the stall. Measurements were extracted from the app as a text file and sent to the statistician to be blindly analysed.

Ethogram

Video recordings were sent to a certified ethologist to be blindly reviewed. Ethograms were used to confirm the mechanism of stress induction. Table 1 describes the behavioural variables that were registered during this study.

STATISTICAL ANALYSIS

Firstly, results are described as the mean and standard deviation of the ethogram score and heart rate during the experimental phase and during the resting phase. We utilised the Wilcoxon Signed Rank test to test whether there is a significant change in the measurements between Resting and Experiment. We utilised the Wilcoxon Signed Rank test form both groups in order to be able to compare the results from the ethogram and heart rate measurements.

We further tested the correlation between the experimental ethogram and heart rate through Spearman's rho, which is a non-parametric correlation testing method. To test the correlation between the resting ethogram and heart rate, we used Spearman's rho. Further we tested the regression between the experimental ethogram scores and heart rates.

RESULTS AND DISCUSSION

Heart rate

Looking at the descriptive of our measurements, we are able to observe an increase in the mean and standard deviation of the experimental heart rate data ($M = 99.65$, $SD = 16.19$) compared to the resting heart rate ($M = 34.69$, $SD = 4.41$) (Table 2).

Similarly, the Wilcoxon Signed Rank test also confirms that there is a statistically significant difference between the resting and experimental heart rate measurements ($Z= -6.093$, $p < .001$) (Table 3).

Ethogram

Looking at the descriptive of our measurements, we are able to observe a noticeable increase in the mean and standard deviation of the experimental ethogram score ($M = 21.33$, $SD = 6.62$), compared to the resting ethogram ($M = 4.82$, $SD = 1.91$) (Table 4).

The Wilcoxon Signed Rank test show that there is a statistically significant difference between, the measurements of the ethogram in the resting and experimental phases ($Z = -6.095$, $p < .001$) (Table 5).

Table 1

Studied behaviours and interpretation of each behaviour are described

Studied behaviour	Code	Description
Head position	HP	High neck and head position and/or head throwing up/down repeatedly and rapidly
Ear movement	EM	Sudden and/or increased ear movement and/or ears flattened posteriorly.
Front leg pawing	LP	Foreleg extended anteriorly then drawn back, dragging ventral toe of hoof along ground repetitively
Nostrils dilatation	ND	From semi-flattened nostrils become circular
Vocalisation	VA	Any high-pitched vocalisation coming from the mouth
Overall posture	OP	Tense posture with all four legs on the ground or nervous movements, quivering,

Table 2

Mean and standard deviation of resting and experimental heart rate

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation
Resting HEART RATE	49	28	43	34.69	4.417
Experimental HEART RATE	49	65	127	99.65	16.191
Valid N (listwise)	49				

Table 3

Experimental and resting heart rates differ significantly

Test Statistics[a]

	Experiment HEART RATE - Resting HEART RATE
Z	-6.093[b]
Asymp. Sig. (2-tailed)	.000

a. Wilcoxon Signed Ranks Test
b. Based on negative ranks.

Table 4

Mean and standard deviation of resting and experimental ethogram scores

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation
Resting ETHOGRAM	49	2	8	4.82	1.911
Experimental ETHOGRAM	49	10	33	21.33	6.619
Valid N (listwise)	49				

Table 5

Experimental and resting ethogram scores differ significantly

Test Statistics[a]

	Experimental ETHOGRAM - Resting ETHOGRAM
Z	-6.095[b]
Asymp. Sig. (2-tailed)	.000

a. Wilcoxon Signed Ranks Test
b. Based on negative ranks.

Table 6

Correlations between the experimental ethogram and heart rate

Correlations

			Experimental ETHOGRAM	Experiment HEART RATE
Spearman's rho	Experimental ETHOGRAM	Correlation Coefficient	1.000	.309*
		Sig. (2-tailed)	.	.031
		N	49	49
	Experiment HEART RATE	Correlation Coefficient	.309*	1.000
		Sig. (2-tailed)	.031	.
		N	49	49

*. Correlation is significant at the 0.05 level (2-tailed).

Table 7

Correlations between the resting ethogram and heart rate

Correlations

			Resting ETHOGRAM	Resting HEART RATE
Spearman's rho	Resting ETHOGRAM	Correlation Coefficient	1.000	.238
		Sig. (2-tailed)	.	.100
		N	49	49
	Resting HEART RATE	Correlation Coefficient	.238	1.000
		Sig. (2-tailed)	.100	.
		N	49	49

Table 8

Regression between experimental ethogram scores and heart rates

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	239.932	1	239.932	6.054	.018[b]
	Residual	1862.844	47	39.635		
	Total	2102.776	48			

a. Dependent Variable: Experimental ETHOGRAM
b. Predictors: (Constant), Experiment HEART RATE

Table 9

Correlations between the difference in heart rates and ethograms

Correlations

		Difference in Heart Rate	Difference in Ethogram
Difference in Heart Rate	Pearson Correlation	1	.292*
	Sig. (2-tailed)		.042
	N	49	49
Difference in Ethogram	Pearson Correlation	.292*	1
	Sig. (2-tailed)	.042	
	N	49	49

*. Correlation is significant at the 0.05 level (2-tailed).

Heart rate and ethogram correlations

When comparing the results of the experimental ethogram and heart, there is a significant positive correlation between the ethogram scores and heart rate (r (47) = .31, p = .031) (Table 6).

When comparing the results of the resting ethogram and heart rate, there is no significant correlation between the resting ethogram and heart rate (r (47) = .29, p = .100) (Table 7).

The test of regression between the experimental ethogram scores and heart rates shows a significant, however overall, rather weak regression between the ethogram score and heart rate in the experimental condition (R^2 = .114, F (1, 47) = 6.054, β = .338, p=.018) (Table 8).

Further, when testing the correlation,the size of the difference in the ethogram score (experimental ethogram minus resting ethogram) and heart rates (experimental heart rate minus resting heart rate), we are able to show that the difference in ethogram measurements is correlated to the difference in the heart rates (r (47) = .29, p = .05) (Table 9).

Firstly, the Wilcoxon Signed Rank tests clearly confirm that there exists a difference in the heart rate of our sample between the initial resting condition and the post-experiment condition. This, paired with the increase in mean heart rate of the group from the resting to experimental conditions, confirms that when introducing our sample to a situation of stress, the heart rate of horses increases. Secondly, the Wilcoxon Signed Rank test also confirms the difference between the ethogram scores of our sample in the resting and experimental conditions. Further, the mean ethogram score has clearly increased from the resting condition after the removal of the foal. These can be understood to confirm that acute stress was induced by removing the foal. Overall, these results show, that while in the resting condition, ethogram measurements and the heart rate are not necessarily associated. In a state of stress, both the ethogram measurements and the heart rate show a certain degree of association, in the sense,that a higher heart rate in the moment of stress, may be associated with a higher ethogram score, showing that acute stress could be assessed by heart rate in horses.

Discussion

This study provides valuable insight into the indicators of acute stress in equines. By understanding the causes and indicators of stress, caretakers can implement strategies to mitigate stress and promote the overall welfare of these animals. Regular monitoring and adapting care practices based on individual needs are key to ensuring that equids lead healthy and stress -free lives. Regular monitoring of stress indicators in individual animals can help in timely intervention and management. This requires nonetheless the use of non-invasive techniques but is also accessible. The significant increase in heart rate observed in response to the stressor aligns with findings of previous studies (4). Our study aimed to simplify the evaluation of stress by focussing only on the measurement of the heart rate (HR). Using accessible devices such as Polar Heart Rate H10 monitors, stress can be measured instantly, accurately, and can be recorded even by non-professionals. The results of this study reveal the positive correlation between the stress ethogram and the increase in heart rate in mares when temporarily separated from their foals, thus showing that the heart rate alone is a reliable stress indicator when measured with a PolarH10 sensor attached to a chest strap. Moreover, the increase in heart rate was greater than the increase in ethogram score when comparing non-stressed mares to stressed mares, further showing the reliability of heart rate alone as a stress indicator. When removing the foals from their dams, most mares did not intensify their locomotion behaviour they moreover remained still looking for the foal that had departed, performing rather static stress behaviours; thus,locomotion did not influence the increase of heart rate in this case. Results demonstrate a positive correlation between the ethogram measurements and the heart rate during stressful episodes (p= .018), showing that the higher heart rate in the moment of stress is associated with a higher ethogram score. The consistency of the results validates that the two parameters can be used independently to measure the level of emotional excitability in horses. Heart rate measurement thus becomes effective and handy for assessing the emotional stress of horses at various times.

CONCLUSIONS

The study points to the potential of further research in the area of equine stress. Further studies are needed to assess whether heart rate could be a stress indicator during training or competition. Additionally, exploring the stress response of horses related to housing and management techniques might reveal insights into horse behaviour and emotions. This study confirms that heart rate is a reliable acute stress indicator in equines. Understanding this is essential to promoting horse welfare and better management techniques, thus supporting the health and performance of horses.

Authors' contribution

The idea for the study was conceived by Dan Manolachescu and Ionel Papuc.

The data collection and analysis were designed by Mirela Tripon, Zsofia Daradics, Cristian Crecan.

The article was written by Dan Manolachescu , with contributions from Zsofia Daradics and Tripon Mirela.

Ethical Considerations

This study was carried out with authorisation and completion of an informed consent by the horse owners, and approved by the Ethics Committee for Animal Experimentation of the University of Agricultural Sciences and Veterinary Medicine in Cluj-Napoca (protocol No. 371/07.04. 2023).

Conflict of Interest

The authors declare no conflict of interest.

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