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SALIVARY CORTISOL LEVELS IN HORSES AND THEIR RIDERS DURING THREE-DAY-EVENTS

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Abstract

The group of 36 warm-blooded half-bred horses (18 stallions and 18 mares) and their riders (20 men and 16 women), who ended three-day-events, were selected for the study. The horses were aged 4 to 6 years, while the riders were 19 to 34-year-old. The saliva samples were collected after each phase of the competitions. The cortisol concentration was determined using an immunoassay method. The following factors were considered: type of competition, horse sex, and rider gender. In horses, the statistically important correlation was found between the results obtained for the dressage and cross-country, for the cross-country and show jumping, and for the dressage and show jumping. An analogous comparison for the riders suggests a statistically significant correlation between the data obtained for the cross-country and show jumping. Comparing the data of horses and their riders, a significant correlation coefficient was found for the cross-country group of woman and the dressage group of men. In conclusion, the salivary cortisol level in individual horses in each phase of three-day-event was found to be repetitive. Therefore, the salivary cortisol test is demonstrated to be a useful method to evaluate the horse response to each type of competition during three-day-events.

Key words: horses, riders, saliva, cortisol, three-day-events.

Interactions between humans and horses can expose the horse to various stressful situations. This includes veterinary procedures (1), social isolation (8), physical, and mental training (4, 10, 18), participation in equestrian competitions (3, 20). Direct human-horse relationship also significantly influences the horse emotional arousal. Horses are known to be sensitive to human presence and to human behaviour (9) including the body language used by a trainer (7). Such stressful situations increase emotional arousal that can change the horse behaviour and, hence, negatively influence its performance, welfare, and human safety. In recent years, the stress level has been evaluated on the basis of cortisol concentration in saliva (11, 15). In fact, a high correlation coefficient found between blood serum and salivary cortisol concentrations validates saliva-sampling as a useful technique for cortisol release in both humans and horses (2, 5, 16, 21). In humans, mental stress caused by participation in sport competitions was found to increase salivary cortisol level substantially (14). Additionally, in horses, the salivary cortisol levels were found to reflect the stress level not only during various equestrian competition events (15, 16, 20) but also during training and road transport (18, 19). However, it is known, that not only stress, but also physical activity is a factor, which increases the hypothalamus-pituitary-adrenal axis and cortisol release (4).

The aim of the study was to compare the salivary cortisol levels in horses and their riders during three-days-event competitions.

Material and Methods

Horses and riders. The study took place during the Polish Horses Championships for Young Horses in Three-Day-Events in Bialy Bor. Among 42 warm-blooded half-bred horses, which took part in these competitions, 36 horses and their riders, were taken for the study. According to the age, the horses competed in three age-related groups: 1) 4-year-old horses (eight stallions and eight mares), 2) 5-year-old horses (four stallions and six mares), and 3) 6-year-old hoses (six stallions and four mares). Detailed data concerning the conditions of following phases of competitions for

subsequent age-related groups of horses are shown in Table 1. None of the horses had any clinical symptoms of health disorders.

The 36 riders, 20 men and 16 women, were 19 to 34-year-old. Each rider had trained and competed with his or her own horses for at least 10 month prior to the study.

The whole study, accepted by the Local Ethics Review Committee for Animal Experimentation, was conducted according to the European Community regulations concerning the protection of experimental animals.

Saliva sampling. For each horse, three saliva samples were collected: 1) 5-7 min after the dressage, on the 1st d of the events, 2) 5-7 min after the cross-country, on the 2nd d of the events, and 3) 5-7 min after the show jumping, on the 3rd d of the events.

The saliva samples were collected with a small piece of sponge, which was inserted into horse's mouth using tweezers. The riders were instructed to place and hold the sponge in their mouths until it became saturated. After soaking in saliva, the sponge was placed in a plastic tube, as described previously (20) and frozen.

Analyses. Before the examination, the saliva samples were melted, warmed up to room temperature, and centrifuged at 500 × g for 15 min at room temperature. Afterwards, the sponge was removed and the saliva was transferred to test tubes.

The concentration of cortisol in saliva samples was measured by enzyme-immunoassay method using the CORTISOL EIA kit DSL-10-67100 (Diagnostic System Laboratories Inc., USA). The minimal detectable concentration was 0.011 µg/dL (0.3 nmol/L). The absorbance was measured using a Multiscan reader (Labsystem, Finland) equipped with a GENESIS V 3.00 programme. The results were expressed as nmol/L.

Statistics. The results including standard deviation (SD) were analysed using the common SAS programme and the univariate ANOVA method for analysing repeated measures and Tukey test. ANOVA method rather than ANOVA-GLM (13) was chosen because of the high variation of the investigated values. The following factors and effects were considered: type of competition, horse sex, and rider gender. The Pearson correlation coefficient was also assessed to compare the analysed data. The statistical significance was accepted at the level of $P \leq 0.05$.

Results

The results of the salivary cortisol concentration in horses are shown in Table 2. The obtained data did not differ statistically. The values of the correlation coefficient counted between the results obtained in horses in subsequent phases of the study are shown in Table 3.

Table 1
Technical conditions of subsequent phases of tree-day events for age-related groups of studied horses

Technical conditions and parameters of obstacles in subsequent phases of competition	4-year-old horses	5-year-old horses	6-year-old horses
Dressage			
Time of presentation (min)	4	4	4
Class of programme	CNC L	CIC*1A	CIC A
Cross-country			
Distance (m)	1,600-2,000	1,600-2,800	2,400-3,200
Number of jumps	15-20	17-25	20-25
Speed (m/min)	450-500	470-520	520
High of the solid part of obstacles (cm)	100	105	110
Full high of the obstacles (cm)	110	120	130
Wide of the higher point of obstacles (cm)	110	120	140
Wide of the basis of obstacles (cm)	160	180	210
Wide of plain obstacles (cm)	150	200	280
Maximal high of landing sites (cm)	120	140	160
Show jumping			
High of the obstacles (cm)	100	105	150
Wide of the obstacles (cm)	100	120	140
Wide of the basis or triple bares (cm)	130	160	190
Numbers of obstacles/jumps	8/9	8-10/12	10-11/13
Distance (m)	300-400	350-400	350-450
Speed (m/min)	300	300	350

CIC - Concourse International Combine, CNC - Concourse National Combine

Table 2
Salivary cortisol concentration in the studied horses (means ± SD, nmol/L)

Time of saliva sampling	Stallions (n = 18)	Mares (n = 18)	All horses (n = 36)
after dressage	3.52 ± 2.22	4.72 ± 4.28	4.11 ± 2.99
after cross-country	5.79 ± 4.32	6.78 ± 6.54	6.27 ± 4.98
after show jumping	5.01 ± 2.87	5.98 ± 4.89	5.48 ± 3.09

Table 3
Correlation coefficient between the salivary cortisol concentrations determined in subsequent phases of the study in horses

Time of sampling	after dressage	after cross-country
after cross-country	0.382*; P=0.023	-
after show jumping	0.596*; P<0.001	0.662*; P<0.001

* - Values are statistically significant

Table 4
Salivary cortisol concentration in the studied riders (means ± SD, nmol/L)

Time of saliva sampling	Men (n = 20)	Women (n = 16)	All riders (n = 36)
after dressage	42.4 ± 22.2 $^{a\,x}$	39.0 ± 21.0 $^{a\,x}$	42.5 ± 18.9 ^{a}
after cross-country	72.3 ± 38.3 $^{b\,x}$	53.2 ± 21.2 $^{b\,y}$	64.0 ± 34.0 ^{b}
after show jumping	43.6 ± 22.6 $^{a\,x}$	34.7 ± 18.1 $^{a\,x}$	40.6 ± 17.9 ^{a}

$^{a, b}$ - data in columns with different superscripts differ significantly at P≤0.05
$^{x, y}$ - data in rows with different superscripts differ significantly within groups at P≤0.05

Table 5
Correlation coefficient between the salivary cortisol concentrations determined in subsequent phases of the study in riders

Time of sampling	after dressage	after cross-country
after cross-country	-0.009; P=0.96	-
after show jumping	-0.033; P=0.85	0.377*; P=0.029

* - Values are statistically significant

Table 6
Correlation coefficient between riders' and horses' salivary cortisol concentrations determined in subsequent phases of competitions

Time of sampling	All riders (n = 36)	Women (n = 16)	Men (n = 20)
after dressage	0.347; P=0.052	-0.069; P=0.82	0.419*; P=0.046
after cross-country	0.195; P=0.26	0.713*; P=0.012	-0.013; P=0.89
after show jumping	0.161; P=0.35	0.496; P=0.054	-0.055; P=0.09

* - Values are statistically significant

A statistically important correlation was found between the results obtained for the dressage and cross-country, for the cross-country and show jumping, and also for the dressage and show jumping. The cortisol concentrations measured in saliva samples taken from riders are presented in Table 4. The results obtained for the samples after cross-country were significantly higher than those obtained after dressage and after show jumping. Moreover, the correlation coefficient found between the data obtained in riders for the cross-country and show jumping was statistically significant (Table 5). Comparing the horses and their riders (Table 6), a statistically significant correlation coefficient was found for the cross-country group of woman and the dressage group of men. High but statistically dubious correlation coefficients were also obtained for the show jumping group of women (P=0.054) and all the dressage riders (P=0.052).

Discussion

In the studied horses, participation in the competition caused cortisol release; however, the changes in the mean salivary cortisol concentration from group to group did not reach the level of statistical significance. Probably, the relatively small number of horses, and the high individual variability of salivary cortisol levels from horse to horse, which is to be expected, decreased the possibility of observing statistically significant differences. High variability of salivary cortisol level from horse to horse was also observed by other researchers (16, 20).

The intensity of the horse's response to the studied stimuli, which is rooted in its individual traits is very important because it can be used in practice. Namely, the cortisol release rate caused by dressage alone influenced the similar rate caused by cross-country and show jumping in the next 2 d. Therefore, the horse response to cross-country or show jumping could be predicted on the basis of the salivary cortisol data taken after dressage on the 1st d of the events, which can simplify future tests.

In riders the participation in cross-country competitions involved higher cortisol release than in dressage and show jumping. This is probably because cross-country lasts longer than the other two events. It is known that cortisol release is proportional to the duration of the exercise (4). The participation in cross-country, also requires the overcoming of solid obstacles, which is dangerous and therefore could be stressful.

As it turned out, a rider gender effect was observed either. Namely, the cortisol release was higher in men than in women during participation in cross-country. This observation is consistent with the results of other studies, in which males showed greater cortisol response to stress than females (6).

The cortisol release in riders expectedly exhibits great individual variation, which was also observed in other studies (12). In the study, data obtained for cross-country and show jumping correlated positively. This is understandable because these two events emphasise the rider's physical effort and mental commitment, whereas dressage is much less exhausting.

The analysis of the horse-rider interaction suggests an interesting correlation between the male rider and his horse salivary cortisol levels for the dressage, and separately between female rider and her horse for the cross-country. This finding suggests that the human-horse interaction can be influenced by the rider gender and by the type of competition. Such gender-dependent differences in cortisol release in response to various psychophysical loads in humans were reported previously (6). Moreover, an increase in human emotional arousal and nervousness influenced the horse emotional reactions (9, 17). Significant correlations were found also between horse and rider salivary cortisol levels during show-jumping (15). This is confirmed by a significant correlation found between the horse and human cortisol release in the study. While such human-horse interaction effects are intuitively agreeable and broadly discussed especially in the context of sympathetic training methods (10), such hypotheses require more research to be undertaken.

In conclusion, individual horses are found to vary greatly in their intensity of cortisol release in response to participation in an event. In the context of such individual variability, the horse response to each phase of a three-day-event is found to be repetitive. Therefore, the salivary cortisol test seems to be a useful and efficient method to evaluate the horse response to competitions including the influence of the rider.

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