R. Bras. Zootec., 49:e20190142, 2020
https://doi.org/10.37496/rbz4920190142

Biometeorology and animal welfare
Full-length research article

Revista Brasileira de Zootecnia

Brazilian Journal of Animal Science
e-ISSN 1806-9290
www.rbz.org.br

Frequency and intensity of pain symptoms detected during classic massage sessions of selected body parts in purebred Arabian racing horses

Izabela Wilk[1], **Iwona Janczarek**[1*], **Witold Kędzierski**[2], **Agnieszka Pomorska**[3]

[1] University of Life Sciences in Lublin, Department of Horse Breeding and Use, Lublin, Poland.
[2] University of Life Sciences in Lublin, Department of Biochemistry, Lublin, Poland.
[3] University of Life Sciences in Lublin, Department and Clinic of Animal Internal Diseases, Lublin, Poland.

***Corresponding author:**
iwona.janczarek@up.lublin.pl

Received: July 12, 2019
Accepted: May 17, 2020

How to cite: Wilk, I.; Janczarek, I.; Kędzierski, W. and Pomorska, A. 2020. Frequency and intensity of pain symptoms detected during classic massage sessions of selected body parts in purebred Arabian racing horses. Revista Brasileira de Zootecnia 49:e20190142. https://doi.org/10.37496/rbz4920190142

ABSTRACT - We analysed the frequency of symptoms and degree of muscle pain in selected body parts of racing horses assessed during classic massage sessions. The influence of horse's sex on obtained results was considered. The potential for the early determination of pain in horses by analysing their behaviour and cardiac parameters during a massage session was also evaluated. The study was conducted on 20 three-year-old purebred Arabian horses during one racing season. In the racing season, cyclic classic massage sessions were performed, during which the frequency of symptoms and the degree of pain in the neck, back, croup, front limbs, and hind limbs were analysed. A behavioural assessment of the horses was conducted, and cardiac parameters were analysed. During massage, the frequency of pain symptoms in front limbs amounted to 26, while in croup, it did not exceed 6. The studied horses were most susceptible to pain in the front limbs and in the back, with greater severity in stallions than in mares. An assessment of the frequency and severity of pain symptoms should not be based on changes in behaviour of horses or on cardiac parameters (HR and LF:HF ratio) during massage sessions. However, these methods can be applied after pain reactions intensify. Meanwhile, qualified masseurs can diagnose slight muscle pain during massage sessions.

Keywords: back lesions, behaviour, heart rate variability, horse

Introduction

Injuries to the locomotor system represent a common health problem in racing horses (Rossdale et al., 1985; Kobluk et al., 1990; Lindner and Dingerkus, 1993; Bailey et al., 1999; Perkins et al., 2005a b). Tendon strains and joint sprains and fractures are occupational hazards in these animals and the main causes of many pathological conditions or even death (Clegg, 2011). This triggers negative emotions in many people for whom the maintenance of the good welfare of the horse represents a challenge, especially for the youngest animals, namely two- or three-year-old horses (Cogger et al., 2006; Clegg, 2011). In horses, injuries generate economic losses to the equestrian industry due to increased treatment expenses and the loss of opportunity to earn money on the race track (Perkins et al., 2005a). Unfortunately, it is not always possible to determine the exact cause of an injury, and sometimes the lesions are neglected or even overlooked at the subclinical stage (Dalglish and Rick, 2011). In horses, typical disease symptoms may not be recognised as reasons for reduced performance or gait abnormalities. A horse trainer often blames the temperament of a horse for poor racing results (Geor, 2001), thus missing the actual cause of the indisposition of its body. Sometimes stiffness is considered as due to pain (Taylor et al., 2002).

As reported by Masterson and Reinhold (2014), horses suffer from permanent muscle hypertonia due to daily training and participation in stressful races; this is often only noticed during massage sessions. Massage relaxes tightened muscles and reduces both physical tension and emotional arousal (McBride et al., 2004). In particular, classical massage used in sport and veterinary medicine brings therapeutic effects (Scott and Swenson, 2009). Massage reduces pain symptoms in older horses and helps to maintain the flexibility of the body in stressed individuals, thereby decreasing discomfort (McBride et al., 2004; Palmer, 2014). In the case of race horses, massage supports the cardiovascular system and improves blood supply to the muscles, contributing to better performance results. Massage also has a beneficial effect on locomotor system functioning. Moreover, it has a calming effect on autonomic nervous system activity, which is especially important for racing horses (Kowalik et al., 2017).

During a massage session, the experience of a masseur together with touch contact with the animal should generate accurate information about the prevalence of symptoms and degree of pain. The opinion of a masseur supported with an analysis of physiological, biochemical, and behavioural variables validated as pain markers may provide additional insight into the early diagnosis of injuries based on a few representative characteristics. Therefore, massage could be a diagnostic tool in the early assessment of injuries in racing horses, as determining the health status the animal is sometimes limited to general observations made by stable workers and a possible examination by a veterinarian, although only when clinical symptoms are present.

Pain also induces changes in cardiac parameters (heart rate variability, HRV) in horses (Rietmann et al., 2004; Bussières et al., 2008). Therefore, the monitoring of HRV parameters has been used as a useful and non-invasive method of pain evaluation in horses (Rietmann et al., 2004; Erber et al., 2012; Diego et al., 2016). However, many other factors can influence HRV parameters (Tarvainen et al., 2014). For example, some breed- and sex-related differences were found in HRV parameters recorded in emotionally aroused horses (Janczarek and Kędzierski, 2011; Wilk et al., 2015). Thus, the measurement of HRV as a method to evaluate pain intensity in individuals is not used in practice.

These points are covered by the first objective of the paper, which was to determine the frequency and intensity of muscle pain in particular body parts of purebred Arabian racing horses, using classical massage as a method for pain detection. The second objective was to explore the potential for the early determination of pain in horses by analysing their behaviour and cardiac parameters during a massage session. The influence of the sex of horses on achieved results was also analysed.

Material and Methods

The animal care and experimental procedures were in accordance with the European Committee Regulations on Protection of Experimental Animals and were approved by the Local Ethic Review Committee for Animal Experiments (ref. no. 78/2012).

At the beginning of the experiment, 24 horses were included in the study (12 stallions and 12 mares). During the racing season, three mares and one stallion were eliminated *inter alia* because of injuries. Finally, the study was conducted on 20 three-year-old purebred Arabian horses subjected to race training. During the study, the horses were kept in the Tor-Służewiec race track facilities in Warsaw (Poland), in the racing stable led by only one trainer. The horses were regularly exercised for about 1 h a day, six days a week. The riders rode the horses at a walk and trot for approximately 10 min as a warm-up exercise. After a 10-min warm up, the horses galloped on the sand track at a speed of 6-12 m/s, covering a distance of 1200-1800 m. The speed and duration of the exercise were individually adapted to the performance level of each horse. After exercise, the horses were cooled down in an automatic horse walker for 30 min.

All studied horses had the same training load during each period and had the same number of races. None of the horses had any major breaks in training greater than two or three days. The two- or three-day rests in training routine happened only once or twice a season.

The 20 studied horses were trained and participated in races from the beginning until the end of the study period. Except for any feelings of pain detected by the masseur, the horses did not show clinical symptoms of any illness, as determined by the trainer and the two authors who are veterinarians specialised in horse illnesses.

Before the study, the horses had never been massaged. During the experiment, a classical relaxing massage was applied to each of the studied horses, and the results of the scheduled examinations were collected. The horses were massaged by two experienced masseurs who had appropriate professional qualifications (course completion documents and excellent references from horse owners). Massage sessions were performed three times a week (Mondays, Wednesdays, and Fridays) and lasted from 30 to 60 min a day per horse, depending on the individual needs of each horse. Namely, at the beginning of the study, the horses needed more time to accept the touch and other activities of the masseur; thus, masseurs spent at least 60 min while working with a horse. In the following months, from time to time, muscle tensions occurred in some horses. Therefore, they needed more time to relax, and massage sessions lasted 45-60 min. For each session, the horses were randomly assigned to the masseurs. Each massage session proceeded as follows: first, the neck was massaged, followed by the cranial part of the trunk with an emphasis on the scapula, and then the middle part of the horse, focusing on the back muscles. When the back and chest area had been massaged, the croup area was covered, and finally, the front and hind limbs were treated (details were described by Kowalik et al., 2017). A typical classical relaxing massage session obligatorily includes the massage of the following muscles and tendons:

the neck: brachiocephalicus, cervical rhomboideus, cervical serratus ventralis, and trapezius (cervical part);

the back: longissimus and latissimus dorsi;

the croup: medial gluteal, semitendinosus, semimembranosus, and biceps femoris;

front limbs: supraspinatus, infraspinatus, biceps and triceps brachii, radial carpal extensor, and interosseous;

hind limbs: quadriceps femoris, gastrocnemius, long digital extensor, and superficial digital flexor tendon.

Each massage session was observed by one of the two authors, and each masseur was equipped with a voice recording device. During each massage session, the existence and severity of pain symptoms in the massaged body parts was determined. Masseurs made detailed notes (first as a voice record on the recorder, then in paper form) on pain symptom prevalence, the affected part(s) of the horse body and scores on the scoring scale (Table 1) for each massaged region. Pain symptoms, which were considered separately, were recorded from the moment when symptoms appeared until they ceased. To evaluate pain severity, the masseurs used a scoring scale supplied by the authors. A five-point scale was used, which focussed on pain severity during a classical massage session. The duration of pain symptoms was not considered.

Scores were also assigned to the horses for their behaviour during massage sessions. The scores were given by one of the two authors, who observed a randomly chosen horse-masseur pair and noted the horse responses, based on a prepared score scale (Table 2). The authors were well experienced in evaluating the behaviour of horses with the use of a point score system thanks to the use of this method

Table 1 - Degree of muscle pain severity in the selected body parts

Score	Behavioural manifestations in horses
1	No sensibility symptoms, tissue insensitive to touch, muscles remain relaxed when palpated
2	Tissue minimally sensitive to touch, muscle tension ceases within seconds after massage is started
3	Horse has mild discomfort on palpation and may present short and rapid muscle contractions when palpated
4	Horse prevents it from being palpated by shifting its body forward, backward, or laterally
5	Horse presents attempted bite, scuttering, prancing, or other violent response in an attempt to prevent palpation

earlier (Janczarek et al., 2017). The five-point score system was mainly based on the response of the horses to the massage of individual body parts and the general wellness of the horse.

Cardiac parameters were also measured during massage sessions with a Polar S800CX telemetric unit (Polar OY, Finland), which consisted of an electrode with a transmitter attached at the heart level and a receiver recording the data in a microcomputer. The data were then loaded into the main computer with a peripheral IrDA unit. Results were analysed with Kubios HRV software (Kuopio, Finland) (Tarvainen et al., 2014). The following two parameters were analysed: heart rate (HR), i.e., the number of heart beats per minute (bpm), and frequency domain analysis covering the ratio of the low frequency component (LF: 0.04-0.15 Hz) to the high frequency component (HF: 0.16-0.4 Hz). The LF reflects a shift towards sympathetic activity of the autonomic nervous system, whereas the HF is mainly attributable to the parasympathetic (vagal) activity. Thus, the LF:HF ratio is used as a reflection of sympatho-vagal balance (Rietman et al., 2004; von Borell et al., 2007). Synchronisation of a manual stopwatch with the stopwatch of the receiver and note-taking during massage sessions permitted the assignment of consecutive fragments of data to the massage of the body parts.

During the present study, the horses were treated with a classical massage over a six-month period. During this period of time, their pain and behaviour were assessed, and HRV parameters were recorded 81 times in each horse. To simplify the analysis and presentation of this large amount of data, it was necessary to group the results into time periods. Based on data included in other studies (Stachurska et al., 2015; Janczarek et al., 2016; Kowalik et al., 2017), we decided to analyse data in three two-month periods. Moreover, these three periods covered the three phases of the race season: the beginning, when the horses habituate to participation in the races; the middle, when the most important races occur; and the end, including the last races which decide the final placing of each horse in general race performance.

All data collected during massage sessions were grouped according to the following time frames: first period, from 10th May to 10th July; second period, from 11th July to 10th September; and third period, from 11th September to 11th November.

This led to 27 measurements of the degree of pain in the selected body parts, behavioural assessments, and cardiac parameters for each horse in each of the race season periods.

Before the statistical analysis method was chosen, data were checked for normal distribution using the Shapiro-Wilk, Kolmogorov-Smirnov, Chi-square, and Lilliefors tests and probability (quantile-quantile) plots. In all cases (including discrete data) these tests demonstrated that the data followed a normal distribution; this was probably due to the large sample size. Therefore, the obtained results were analysed by the analysis of variance with SAS (Statistical Analysis System, version 6.0) using the General Linear Mean and Mixed models. The statistical model for objective data (frequency of pain symptoms, degree of pain severity, HR, and LF:HF ratio) included the random effect of the horse and fixed effects of the periods of time (1-3), sex of the horse (stallion, mare), body part (neck, back, croup, front limbs, hind limbs), and masseur (1-2). The statistical model for

Table 2 - Specific behaviours of the horses during massage sessions and scoring system

	Behavioural attitude of horses
1	Horse cannot be approached or massaged, motoric activity in response to presence of masseur (shifting the body sideways away from the person or trying to push sideways on the person, rearing), aggressive behaviour in response to the touch of the masseur (an attempt to kick and/or bite)
2	Horse hinders massaging of its whole body, defensive reactions (high head raising, shifting the body away from the hands of the masseur, horse paws the ground), or harmless negative attitude (marked neck tightness, head high, beating with the tail)
3	Horse hinders massaging of some body area, defensive reactions, or harmless negative attitude only during touching specific place; the horse looks at the masseur
4	Horse presents a harmless negative attitude when some body parts are massaged, generalized relaxation of the body, head lowered
5	Horse voluntarily approaches a masseur, loosening the whole body, head low, relaxed attitude

behavioural scores analysed separately included fixed effects of the period of time (1-3), sex of the horse (stallion, mare), and observer (1-2). The interactions between main effects were tested and found statistically insignificant. The adjustment of the statistical model (R) was always above 70%. Differences between the levels of effects investigated were tested by post-hoc testing comparisons of least squares means (LSM) using Duncan's test. Correlations between the variables were analysed with Pearson's correlation. The results are presented as arithmetic means (lsm) with standard errors (SE). The statistical significance was accepted at the level of P≤0.05.

Results

No effect of the masseur factor on the level of analysed features was found. Muscle pain most frequently occurred in the front limbs throughout the experiment, and the least frequently occurred in the neck and in the croup (Table 3). Pain in the croup occurred more frequently in stallions than in mares during the first and second periods of the experiment. The frequency of muscle pain in stallions was lower in the second period than in the other periods, and the frequency of pain in mares was lower during the first period than in the other periods; exceptions were the front limbs of the stallions and the neck and hind limbs of the mares.

In stallions, the severity of pain in the front limbs (over all the periods of the study) and in the back (over the second and third periods) was significantly higher than in the other parts of the body (Table 3). The lowest pain values were observed for the neck, croup, and hind limbs. During the first period of experiment, the severity of pain in various parts of the body of stallions and mares generally did not differ, with the only exception being a lower severity of neck pain in stallions. Back and croup pain in mares during the second and third periods of the experiment was less severe than during the first period. The other values did not differ between the successive periods of the study. The severity

Table 3 - Frequency of pains symptoms and degree of pain severity in the selected body parts in horses during the study

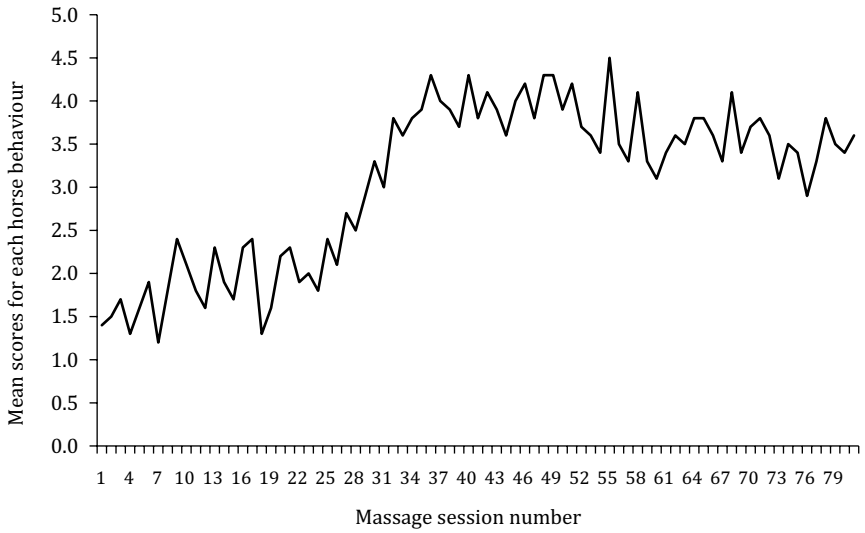
Body part	Frequency of pain symptoms				Degree of pain severity			
	Stallions		Mares		Stallions		Mares	
	lsm	SE	lsm	SE	lsm	SE	lsm	SE
				First period of the study				
Neck	6.44ax	0.02	8.01ax	0.09	2.83ax	0.06	3.61ax	0.05
Back	11.98bx	0.31	9.89ax	0.21	2.81ax	0.09	2.01ax	0.08
Croup	9.31ax*	0.25	4.63bx*	0.13	3.83bx	0.06	3.77ax	0.06
Front limbs	24.63cx	0.75	18.44cx	0.62	1.36cx	0.11	2.28ax	0.08
Hind limbs	16.02bx	0.48	12.33ax	0.42	3.57bx	0.07	3.99ax	0.04
				Second period of the study				
Neck	3.46ay	0.11	4.51ay	0.11	3.27axy	0.06	3.77axy	0.05
Back	8.27by	0.21	9.10bx	0.18	2.29bx	0.05	2.93ay	0.08
Croup	5.49ay*	0.16	9.46by*	0.22	3.52ax*	0.06	4.65by*	0.04
Front limbs	18.97cx	0.59	23.22cy	0.81	2.09bx	0.07	2.83ax	0.07
Hind limbs	10.13by	0.21	11.77bx	0.36	3.93ax	0.06	3.59ax	0.05
				Third period of the study				
Neck	7.63ax	0.22	9.71ax	0.23	4.11ay	0.06	3.98ay	0.06
Back	14.32bx	0.43	15.96by	0.60	2.23bx	0.13	3.25ay	0.07
Croup	10.11ax	0.25	12.30aby	0.37	3.77ax	0.06	4.41axy	0.06
Front limbs	26.41cx	0.86	22.99cy	0.83	2.48bx	0.07	3.67ax	0.08
Hind limbs	17.09bx	0.55	14.61bx	0.49	3.65ax	0.06	4.19ax	0.07

SE - standard error.
Means denoted with different letters for the results of the same body part (a, b, c: comparison of body parts in the same period; x, y: comparison in the subsequent periods; *: comparison of stallions and mares) differ significantly at P≤0.05.

of croup pain in stallions during the second period of the experiment was found to be significantly higher than in mares.

The mean scores assigned to the horses for their behaviour during each massage session are shown in Figure 1. No effect of the observer factor on the level of analysed features was found. Both in stallions and mares, scores were significantly higher in the second and third periods of the study, as compared with the first period (Table 4).

The HR of the horses during the massage sessions usually ranged from 45 to 65 bpm (Table 5). In stallions, the highest HR was observed during the massage of the back and the front limbs during the first and second periods of the experiment. In mares, HR was similar during the massage of various parts of the body in the first period of the experiment. A lower mare HR was recorded during neck massage in the first period and during limb massage sessions in the second period, and a higher HR was recorded during back massage in the third period. Moreover, a significantly lower HR was noted in stallions during the neck massage sessions during the second and third periods of the experiment compared with the first period, and when massaging the front limbs during the first and third



Horses were massaged and their behaviour was scored three days a week during the racing season (81 times); x-axis - the massage session number; y-axis - mean scores assessed for the behaviour of each horse using the five-point scale: 1 - horse shows motoric activity (shifting the body sideways away from the person or trying to push sideways on the person, rearing) and/or aggressive behaviour in response to masseur presence (an attempt to kick and/or bite); 2 - horse hinders massaging: defensive reactions (high head raising, shifting the body away from the masseur' hands, the horse paws the ground); marked neck tightness, head high, beating with the tail); 3 - horse hinders massaging of some body area; defensive reactions or harmless negative attitude only during touching specific place; the horse looks at the masseur; 4 - horse presents harmless negative attitude only when some body parts are massaged; head lowered; 5 - horse voluntarily approaches a masseur; the whole body relaxed; head low.

Figure 1 - Mean scores assigned to the horses for their behaviour during each massage sessions.

Table 4 - Scores assigned to the horses for their behaviour during massage sessions

	First period of the study	Second period of the study	Third period of the study
		Stallions	
lsm	1.25x	4.01y	3.22y
SE	0.05	0.02	0.03
		Mares	
lsm	2.67x	3.61y	3.63y
SE	0.05	0.02	0.03

SE - standard error.
Means denoted with different letters (x, y: comparison in the subsequent periods) differ significantly at P≤0.05; differences between stallions and mares were not statistically significant.

periods compared with the second period. A different pattern was observed in mares. A significantly lower HR was noted during the massage of their croup, as well as the front and hind limbs, during the second and third periods compared with the first period. Stallion HR differed significantly from that of mares during the massage of the neck, back, and croup during the first period and during the massage of the front limbs during the second period. Higher values of these parameters were most frequently noted in mares.

The LF:HF ratio did not differ significantly during the massage of various body parts in the first period of the experiment, neither in stallions nor in mares (Table 5). In stallions, the LF:HF ratio during the massage of the back and hind limbs was significantly higher during the second period of the experiment than during the other two periods. Significant differences in this parameter were also observed during the massage of the front limbs in the third period compared with the second period. In mares, the LF:HF ratio during massage of the croup was significantly higher in the first period than in the following two periods. In mares, the LF:HF ratio during neck massage in the second period was significantly lower than in the first period. Significantly lower values of this parameter were also observed during massage of the front limbs in the third period compared with the other two periods. The LF:HF ratio in stallions differed significantly from that in mares during massage of the croup in the first period of the study and during neck and back massage in the second period; the LF:HF ratio was higher in mares only in the first of these cases.

There was a significant negative correlation between the mean pain severity and the horse behaviour assessment during the massage sessions in the third period of the experiment only (Table 6). There were also significant negative correlations between the mean degree of pain severity and the HR and LF:HF ratio values in the third period of the study (Table 7). The obtained correlation coefficients in range from 0.391 to 0.445 are classified as low according to the scale used in medical research (Mukaka, 2012).

Table 5 - Heart rate and low frequency to high frequency (LF:HF) ratio recorded in the horses during massage sessions

Parameter	Hear rate (bpm)				LF:HF (%)			
Body part	Stallions		Mares		Stallions		Mares	
	lsm	SE	lsm	SE	lsm	SE	lsm	SE
				First period of the study				
Neck	55.67ax*	0.74	44.36axy*	0.59	289.45ax*	7.61	467.01ax*	12.18
Back	65.14bx*	0.68	54.31bx*	0.66	417.66ax	11.48	461.33ax	13.01
Croup	43.60cx*	0.61	57.02bx*	0.86	332.88ax*	10.79	529.17ax*	14.10
Front limbs	55.89ax	0.67	59.66bx	0.87	462.91axy	12.89	455.12ax	13.51
Hind limbs	49.81acx	0.78	54.89bx	0.59	384.54ax	11.50	387.41ax	11.43
				Second period of the study				
Neck	45.06ay	0.58	49.95ax	0.59	546.40ay*	13.54	323.03ay*	10.58
Back	58.88by	0.56	49.22ax	0.61	683.31ay*	16.43	448.27ax*	12.32
Croup	44.72ax	0.47	46.47ay	0.62	504.05ax	18.24	382.11ay	11.79
Front limbs	65.08cy*	0.68	42.88ay*	0.53	547.38ax	18.41	491.29ax	12.30
Hind limbs	45.01ax	0.57	42.11ay	0.61	590.01ay	16.97	439.45ax	12.35
				Third period of the study				
Neck	43.45ay	0.58	40.31ay	0.49	368.39ax	13.22	411.11axy	10.91
Back	48.82ax	0.53	47.93bx	0.55	402.15ax	12.30	389.65ax	11.56
Croup	44.06ax	0.44	38.23az	0.32	365.88ax	13.19	354.32ay	11.43
Front limbs	50.06ax	0.43	42.67aby	0.49	321.05ay	12.29	322.03ay	10.31
Hind limbs	47.67ax	0.61	42.88aby	0.48	414.23ax	13.11	411.28ax	12.27

SE - standard error.
Means denoted with different letters for the results of the same body part (a, b, c: comparison of body parts in the same period; x, y, z: comparison in the subsequent periods; *: comparison of stallions and mares) differ significantly at P≤0.05.

Table 6 - Correlations between mean frequency of pain symptoms and mean degree of pain severity in the massaged body parts compared with the behavioural scores of the horses during massage sessions

Period of the study	Mean frequency of pain symptoms	Mean degree of pain severity
First	−0.179	−0.032
Second	−0.183	−0.105
Third	−0.023	−0.422*

* Significant correlation at P≤0.05.

Table 7 - Correlations between mean frequency of pain symptoms and mean degree of pain severity in the massaged body parts compared with the cardiac parameters

Period of the study	Mean frequency of pain symptoms		Mean degree of pain severity	
	HR (bpm)	LF:HF (%)	HR (bpm)	LF:HF (%)
First	0.152	0.199	−0.279	−0.202
Second	0.098	0.171	−0.129	−0.101
Third	0.125	0.087	−0.391*	−0.445*

HR - Heart rate; LF:HF - low frequency to high frequency ratio.
* Significant correlation at P≤0.05.

Discussion

This study identified massage as a promising tool for the assessment of the incidence and severity of subclinical pain in horses. Moderate to severe muscle pain symptoms (about three and more in the used scale) detected especially in the neck, croup, and hind limbs accompanied some horses during the whole study. According to Walmsley et al. (2010), the lack of treatment of latent ailments leads to prolonged injuries, which results in temporary or permanent exclusion of a horse from use. Simple and effective methods for the assessment of latent ailments in clinically healthy horses would be a helpful tool when training young racing horses; Evans (2007) expressed similar opinions.

The obtained results were presented and analysed in the three two-month periods. Results of other studies indicated that the horse HRV response to innovations in a training routine is long-lasting, namely, the addition of relaxing methods induced visible changes in HRV parameters in the studied horses one to three months later (Stachurska et al., 2015; Janczarek et al., 2016; Kowalik et al., 2017). Moreover, mean scores assigned for the behaviour of horses during each massage session indicated that their response to massage differed significantly after about two months of the study.

Detailed analysis showed that the sex of the horse largely determines the frequency of the occurrence of pain symptoms during the first two months of the racing season. This can probably be attributed to the fact that stallions start intense racing training slightly later than mares, as indicated by Physick-Sheard (1986). The most frequent site of pain in stallions was in the front limbs and in the back, consistent with the results of other similar studies (Jeffcott et al., 1982; Rossdale et al., 1985; Stover, 2003). Racing and sport horses have been shown to suffer from limb injuries after taking part in races or competitions (Stover, 2003). Such injuries usually occur in the flexor tendons of the front limb, sesamoid bones, and carpal joints (Meershoek et al., 2001; Dyson, 2002; Boswell et al., 2003; Stover, 2003). Injuries and ailments of the front limbs are usually caused by an overload on the leading limb while cantering or galloping (McGuigan and Wilson, 2003).

Interestingly, the sex-related differences in the frequency of pain symptoms in selected body parts diminished in the successive months of training. According to Marr et al. (1993), up to 70% of mares end their participation in races because of limb injuries. Thus, monitoring the health status of racing horses on a continuous basis would be advisable, especially in the months with the most intensive training and the greater number of races.

In the current study, severity of pain did not differ between sexes within the selected body parts during the first two stages of the experiment, i.e., the first four months of the racing season. However, in the final stage, the severity of pain was lower in mares than in stallions. This could be because mares participate in races less frequently than stallions during the last months of the racing season. According to Janczarek et al. (2016), protecting mares against overload is required when they are in poor health. It is noteworthy that the most severe pain in horses of both sexes was observed in the front limbs and back region. Back lesions and associated back pain are a common problem in athletic horses (Parkin, 2008; Fonseca et al., 2006; Haussler, 1996). Therefore, these body parts should be closely monitored.

The potential for the early determination of pain in horses by analysing their behaviour and cardiac parameters during a massage session was also considered. However, the correlations of pain symptoms with behavioural scores, as well as with cardiac parameters were insignificant or low. Generally, horses are very sensitive to the human presence and human touch (Baragli et al., 2009), therefore, their response to masseur activity could be individually differentiated. The HR and behaviour of horses depend on their previous experience, e.g., horses treated gently by humans are more relaxed during subsequent contacts with unknown person than horses trained with the use of negative reinforcement (Baragli et al., 2011). Moreover, horses can show elevated HR in response to stressful stimuli despite looking calm while behaviour is being analysed (Baragli et al., 2013). Thus, the reaction of horses differs depending on their previous experience.

On the other hand, the cardiac parameters of horses are considered more objective than behavioural analysis, which explains their role in the study of horses (von Borell et al., 2007). Moreover, they reveal emotions and stress, which do not always have distinct behavioural manifestations (Rietmann et al., 2004; Baragli et al., 2013). These parameters can also be correlated with the severity of pain. Elevated HR and LF:HF ratio values occurred several times during the massage of the body parts that were most frequently and most severely painful, as reported by the masseurs. The scores given by the masseurs to mares were slightly less consistent. However, this phenomenon was apparent in all horses only in the last stage of the experiment, when, for example, the cardiac parameters under observation were seen to increase during back massage. When the physiological parameters and behavioural scores of assessment match, for example, it makes these results more reliable, which was also found in the study conducted by Bachmann et al. (2003).

The considerable number of injuries in racing horses encourages intensive physiotherapeutic treatment, of which massage is the main type (Haussler, 2009; Scott and Swenson, 2009). However, Hesse and Verheyen (2010) found that it is worth preparing horses for such therapy. Both the behavioural assessment and the cardiac parameters measured during the massage sessions indicate that the period during which horses become accustomed to this therapy should last about two months. Only after that period would the response of animals to massage be correct, with cardiac parameters decreasing and remaining at a similar level until the end of the racing season. This situation should not be affected by any pain symptoms. Changes in HR parameters can be attributed to the positive relaxing effect of massage. Similar opinions were expressed by McBride et al. (2004), who found that HR decreased by 4.3% during treatment and by 2.6% after treatment completion. According to the cardiac parameters observed in this study, stallions have a different response to touch than mares, especially during the first period of the study. For example, stallions showed lower values of HR and LF:HF ratio than mares during croup massage, whereas they had higher HR than mares when the back was being massaged. Similar observations were made by Kowalik et al. (2017).

The dual effect of massage (i.e., physiotherapeutic and relaxing) could explain the small number of correlations between the mean frequency and severity of pain and behavioural assessment and cardiac parameters. It turns out that both behaviour and the analysed parameters can be regarded as indicators of pain symptoms only in the final stage of the racing season; at this stage, most of the muscle pain of horses was severe back pain. Similar observations were reported by Fureix et al. (2010), who noted a more frequent occurrence of affective reactions and symptoms of aggression in horses with chronic spinal pain. On the other hand, Rietmann et al. (2004) noted that pain symptoms usually appeared

together with an increase in HR and a more marked response of the sympathetic nervous system compared with the parasympathetic system. Therefore, the behaviour, HR, and LF:HF ratio of horses cannot be used as unambiguous indicators of locomotor pain in horses since they may be caused by other factors that were not analysed.

Conclusions

Results indicate that purebred Arabian racing horses are most susceptible to pain in front and hind limbs and back, with greater severity of pain in stallions than in mares. The sex of horses influenced the frequency of pain symptoms and values of cardiac parameters (heart rate and low frequency to high frequency ratio). An assessment of the frequency and severity of pain symptoms could not be based on changes in behaviour or on cardiac parameters of horses during massage sessions. The potential for the early determination of pain in horses by analysing their behaviour and cardiac parameters during a massage session was rather low; therefore, applying these methods should be investigated more deeply in the future. Meanwhile, qualified masseurs can diagnose slight pain during massage sessions.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: I. Janczarek and W. Kędzierski. Data curation: I. Wilk. Formal analysis: I. Wilk and A. Pomorska. Investigation: I. Wilk. Methodology: I. Janczarek. Project administration: I. Janczarek, W. Kędzierski and A. Pomorska. Software: W. Kędzierski. Supervision: W. Kędzierski. Validation: W. Kędzierski and A. Pomorska. Writing-original draft: I. Janczarek. Writing-review & editing: I. Wilk.

Acknowledgments

The study was supported by the National Centre for Research and Development, Poland (grant N180061).

References

Bachmann, I.; Bernasconi, P.; Herrmann, R.; Weishaupt, M. A. and Stauffacher, M. 2003. Behavioural and physiological responses to an acute stressor in crib-biting and control horses. Applied Animal Behaviour Science 82:297-311. https://doi.org/10.1016/S0168-1591(03)00086-8

Bailey, C. J.; Reid, S. W. J.; Hodgson, D. R. and Rose, R. J. 1999. Impact of injuries and disease on a cohort of two-and three-year-old thoroughbreds in training. Veterinary Record 145:487-493. https://doi.org/10.1136/vr.145.17.487

Baragli, P.; Gazzano, A.; Martelli, F. and Sighieri, C. 2009. How do horses appraise humans' actions? A brief note over a practical way to assess stimulus perception. Journal of Equine Veterinary Science 29:739-742. https://doi.org/10.1016/j.jevs.2009.08.006

Baragli, P.; Mariti, C.; Petri, L.; De Giorgio, F. and Sighieri, C. 2011. Does attention make the difference? Horses' response to human stimulus after 2 different training strategies. Journal of Veterinary Behavior 6:31-38. https://doi.org/10.1016/j.jveb.2010.08.020

Baragli, P.; Vitale, V.; Banti, L.; Bergero, D. and Sighieri, C. 2013. Life experience and object-directed emotions in horses. Journal of Veterinary Behavior 8:e2. https://doi.org/10.1016/j.jveb.2012.12.005

von Borell, E.; Langbein, J.; Després, G.; Hansen, S.; Leterrier, C.; Marchant-Forde, J.; Marchant-Forde, R.; Minero, M.; Mohr, E.; Prunier, A.; Valance, D. and Veissier, I. 2007. Heart rate variability as a measure of autonomic regulation of cardiac activity for assessing stress and welfare in farm animals — A review. Physiology and Behavior 92:293-316. https://doi.org/10.1016/j.physbeh.2007.01.007

Boswell, R. P.; Mitchell, R. D. and Dyson, S. J. 2003. Lameness in the show hunter and show jumper. p.965-975. In: Diagnosis and management of lameness in the horse. Ross, M. and Dyson, S., eds. W.B. Saunders Co., St Louis.

Bussières, G.; Jacques, C.; Lainay, O.; Beauchamp, G.; Leblond, A.; Cadoré, J. L.; Desmaizières, L. M.; Cuvelliez, S. G. and Troncy, E. 2008. Development of a composite orthopaedic pain scale in horses. Research in Veterinary Science 85:294-306. https://doi.org/10.1016/j.rvsc.2007.10.011

Clegg, P. D. 2011. Musculoskeletal disease and injury, now and in the future. Part 1: Fractures and fatalities. Equine Veterinary Journal 43:643-649. https://doi.org/10.1111/j.2042-3306.2011.00457.x

Cogger, N.; Perkins, N.; Hodgson, D. R.; Reid, S. W. J. and Evans, D. L. 2006. Risk factors for musculoskeletal injuries in 2-year-old Thoroughbred racehorses. Preventive Veterinary Medicine 74:36-43. https://doi.org/10.1016/j.prevetmed.2006.01.005

Dalglish, R. A. and Rick, M. C. 2011. Lameness of the Arabian Racehorse: Middle East and North America. p.1057-1061. In: Diagnosis and management of lameness in the horse. 2nd ed. Ross, M. W. and Dyson, S. J., eds. Elsevier Health Sciences, St Louis. https://doi.org/10.1016/B978-1-4160-6069-7.00111-5

Diego, R.; Douet, C.; Reigner, F.; Blard, T.; Cognié, J.; Deleuze, S. and Goudet, G. 2016. Influence of transvaginal ultrasound-guided follicular punctures in the mare on heart rate, respiratory rate, facial expression changes, and salivary cortisol as pain scoring. Theriogenology 86:1757-1763. https://doi.org/10.1016/j.theriogenology.2016.05.040

Dyson, S. 2002. Lameness and poor performance in the sport horse: dressage, show jumping and horse trials. Journal of Equine Veterinary Science 22:145-150. https://doi.org/10.1016/S0737-0806(02)70139-1

Erber, R.; Wulf, M.; Becker-Birck, M.; Kaps, S.; Aurich, J. E.; Möstl, E. and Aurich, C. 2012. Physiological and behavioural responses of young horses to hot iron branding and microchip implantation. The Veterinary Journal 191:171-175. https://doi.org/10.1016/j.tvjl.2011.08.008

Evans, D. L. 2007. Welfare of the racehorse during exercise training and racing. p.181-201. In: The welfare of horses. Waran N., ed. Springer, Dordrecht. https://doi.org/10.1007/978-0-306-48215-1_8

Fonseca, B. P. A.; Alves, A. L. G.; Nicoletti, J. L. M.; Thomassian, A.; Hussni, C. A. and Mikail, S. 2006. Thermography and ultrasonography in back pain diagnosis of equine athletes. Journal of Equine Veterinary Science 26:507-516. https://doi.org/10.1016/j.jevs.2006.09.007

Fureix, C.; Menguy, H. and Hausberger, M. 2010. Partners with bad temper: reject or cure? A study of chronic pain and aggression in horses. PLoS ONE 5:e12434. https://doi.org/10.1371/journal.pone.0012434

Geor, R. 2001. Young horses in training and injury risks. Available at: <http://www.thehorse.com/articles/10065/young-horses-in-training-and-injury-risks>. Accessed on: Mar. 07, 2020.

Haussler, K. K. 1996. The lower back and pelvis of performance horses receive a closer look. Journal of Equine Veterinary Science 16:279-281. https://doi.org/10.1016/S0737-0806(96)80220-6

Haussler, K. K. 2009. Review of manual therapy techniques in equine practice. Journal of Equine Veterinary Science 29:849-869. https://doi.org/10.1016/j.jevs.2009.10.018

Hesse, K. L. and Verheyen, K. L. P. 2010. Associations between physiotherapy findings and subsequent diagnosis of pelvic or hindlimb fracture in racing Thoroughbreds. Equine Veterinary Journal 42:234-239. https://doi.org/10.2746/042516409X478479

Janczarek, I. and Kędzierski, W. 2011. Emotional response to novelty and to expectation of novelty in young race horses. Journal of Equine Veterinary Science 31:549-554. https://doi.org/10.1016/j.jevs.2011.03.012

Janczarek, I.; Kędzierski, W.; Stachurska, A. and Wilk, I. 2016. Can releasing racehorses to paddocks be beneficial? Heart rate analysis – preliminary study. Annals of Animal Science 16:87-97.

Janczarek, I.; Wilk, I.; Kędzierski, W.; Stachurska, A. and Kowalik, S. 2017. Offtrack training ameliorates emotional excitability in purebred Arabian racehorses. Canadian Journal of Animal Science 97:42-50. https://doi.org/10.1139/CJAS-2016-0062

Jeffcott, L. B.; Rossdale, P. D.; Freestone, J.; Frank, C. J. and Towers-Clark, P. F. 1982. An assessment of wastage in Thoroughbred racing from conception to 4 years of age. Equine Veterinary Journal 14:185-198. https://doi.org/10.1111/j.2042-3306.1982.tb02389.x

Kowalik, S.; Janczarek, I.; Kędzierski, W.; Stachurska, A. and Wilk, I. 2017. The effect of relaxing massage on heart rate and heart rate variability in Purebred Arabian racehorses. Animal Science Journal 88:669-677. https://doi.org/10.1111/asj.12671

Kobluk, C. N.; Robinson, R. A.; Clanton, C. J.; Trent, A. M.; Ames, T. R. and Gordon, B. J. 1990. Comparison of the exercise level and problem rate of 95 Thoroughbred horses: a cohort study. p.471-475. In: Proceedings of 36th the Annual Convention of the American Association of Equine Practitioners, Lexington, Kentucky.

Lindner, A. and Dingerkus, A. 1993. Incidence of training failure among Thoroughbred horses at Cologne, Germany. Preventive Veterinary Medicine 16:85-94. https://doi.org/10.1016/0167-5877(93)90078-8

Marr, C. M.; McMillan, I.; Boyd, J. S.; Wright, N. G. and Murray, M. 1993. Ultrasonographic and histopathological findings in equine superficial digital flexor tendon injury. Equine Veterinary Journal 25:23-29. https://doi.org/10.1111/j.2042-3306.1993.tb02896.x

Masterson, J. and Reinhold, S. 2014. Metoda Mastersona – terapia manualna koni. Ed. Galaktyka, Łódź. (in Polish).

McBride, S. D.; Hemmings, A. and Robinson, K. 2004. A preliminary study on the effect of massage to reduce stress in the horse. Journal of Equine Veterinary Science 24:76-81. https://doi.org/10.1016/j.jevs.2004.01.014

McGuigan, M. P. and Wilson, A. M. 2003. The effect of gait and digital flexor muscle activation on limb compliance in the forelimb of the horse *Equus caballus*. Journal of Experimental Biology 206:1325-1336. https://doi.org/10.1242/jeb.00254

Meershoek, L. S.; Schamhardt, H. C.; Roepstorff, L. and Johnston, C. 2001. Forelimb tendon loading during jump landings and the influence of fence height. Equine Veterinary Journal 33(S33):6-10. https://doi.org/10.1111/j.2042-3306.2001.tb05349.x

Mukaka, M. M. 2012. Statistics Corner: A guide to appropriate use of correlation coefficient in medical research. Malawi Medical Journal 24:69-71.

Palmer, S. 2014. Masaż koni. Poradnik dla właścicieli. Popraw zdrowie i samopoczucie swojego konia. Świadome Jeździectwo, Warszawa. (in Polish).

Parkin, T. D. 2008. Epidemiology of racetrack injuries in racehorses. Veterinary Clinics of North America - Equine Practice 24:1-19. https://doi.org/10.1016/j.cveq.2007.11.003

Perkins, N. R.; Reid, S. W. J. and Morris, R. S. 2005a. Profiling the New Zealand Thoroughbred racing industry. 2. Conditions interfering with training and racing. New Zealand Veterinary Journal 53:69-76. https://doi.org/10.1080/00480169.2005.36471

Perkins, N. R.; Reid, S. W. J. and Morris, R. S. 2005b. Profiling the New Zealand Thoroughbred racing industry. 1. Training, racing and general health patterns. New Zealand Veterinary Journal 53:59-68. https://doi.org/10.1080/00480169.2005.36470

Physick-Sheard, P. W. 1986. Career profile of the Canadian Standardbred. I. Influence of age, gait and sex upon chances of racing. Canadian Journal of Veterinary Research 50:449-456.

Rietmann, T. R.; Stauffacher, M.; Bernasconi, P.; Auer, J. A. and Weishaupt, M. A. 2004. The association between heart rate, heart rate variability, endocrine and behavioural pain measures in horses suffering from laminitis. Journal of Veterinary Medicine A, Physiology, Pathology, Clinical Medicine 51:218-225. https://doi.org/10.1111/j.1439-0442.2004.00627.x

Rossdale, P. D.; Hopes, R.; Digby, N. J. and Offord, K. 1985. Epidemiological study of wastage among racehorses 1982 and 1983. The Veterinary Record 116:66-69. https://doi.org/10.1136/vr.116.3.66

Scott, M. and Swenson, L. A. 2009. Evaluating the benefits of equine massage therapy: A review of the evidence and current practices. Journal of Equine Veterinary Science 29:687-697. https://doi.org/10.1016/j.jevs.2009.07.017

Stachurska, A.; Janczarek, I.; Wilk, I. and Kędzierski, W. 2015. Does music influence emotional state in race horses? Journal of Equine Veterinary Science 35:650-656. https://doi.org/10.1016/j.jevs.2015.06.008

Stover, S. M. 2003. The epidemiology of Thoroughbred racehorse injuries. Clinical Techniques in Equine Practice 2:312-322. https://doi.org/10.1053/j.ctep.2004.04.003

Tarvainen, M. P.; Niskanen, J. P.; Lipponen, J. A.; Ranta-Aho, P. O. and Karjalainen, P. A. 2014. Kubios HRV – Heart rate variability analysis software. Computer Methods and Programs in Biomedicine 113:210-220. https://doi.org/10.1016/j.cmpb.2013.07.024

Taylor, P. M.; Pascoe, P. J. and Mama, K. R. 2002. Diagnosing and treating pain in the horse: Where are we today? Veterinary Clinics of North America - Equine Practice 18:1-19. https://doi.org/10.1016/S0749-0739(02)00009-3

Walmsley, E. A.; Steel, C. M.; Richardson, J. L. and Whitton, R. C. 2010. Muscle strain injuries of the hindlimb in eight horses: diagnostic imaging, management and outcomes. Australian Veterinary Journal 88:313-321. https://doi.org/10.1111/j.1751-0813.2010.00597.x

Wilk, I.; Kędzierski, W.; Stachurska, A. and Janczarek, I. 2015. Are results of Crib Opening Test connected with efficacy of training horses in a round-pen? Applied Animal Behaviour Science 166:89-97. https://doi.org/10.1016/j.applanim.2015.02.003