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## Original Research

# Maximum Eye Temperature in the Assessment of Training in Racehorses: Correlations With Salivary Cortisol Concentration, Rectal Temperature, and Heart Rate



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## ABSTRACT

We investigated agreement between eye maximum pixel temperature (using thermography) and rectal temperature (TR) in racehorses, comparing the results with salivary cortisol concentration and heart rate (HR), both at rest and after exercise. Nineteen horses, undergoing training for racing in their first racing season, were studied. Eye maximum pixel temperature, TR, salivary cortisol concentration, and HR were measured before training (BT), within 5 minutes of the end of the training session (T+5), and 2 hours after training (T+120). Eye maximum pixel temperature, TR, salivary cortisol concentration, and HR were all significantly elevated at T+5 compared to BT (all  $P < .001$ ). At T+120, only eye maximum pixel temperature remained significantly elevated compared to BT ( $P < .05$ ). Bland-Altman analysis indicated a poor agreement between eye maximum pixel temperature and TR. We noted no significant correlations among any of the measurements at any time point, with the exception of eye maximum pixel temperature and TR at BT ( $r = 0.55$ ,  $P = .01$ ). In racehorses, eye maximum pixel temperature is a poor estimate of core temperature due to limited agreement with TR. Furthermore, eye maximum pixel temperature is not correlated with accepted measures of stress such as salivary cortisol concentration and HR.

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## 1. Introduction

The reliable measurement of stress in sport horses is important both for animal welfare and optimal sport performance. Both biological factors (e.g., sex, age, breed) and environmental issues influence the physiological stress response in horses [1–4]. A variety of situations to

which sport horses are regularly exposed have been classified as potential stressors. These include training [5,6], mounting by a rider [7], veterinary examination [8], or changes of daily routine [9]. The detection of stress is particularly important for young racehorses, which are put under extreme physical demands in the early stages of their racing careers as their training progresses.

The physiological assessment of stress is typically determined by the invasive measurement of cortisol concentration in plasma. This measurement, however, is a stressor in itself and can lead to confusing results [10]. For this reason, research interest has expanded to identify and

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validate minimally invasive or noninvasive methods of stress measurement in the equine [11].

Techniques to analyze cortisol in equine saliva have now been established, avoiding the need for venipuncture [12,13]. A positive correlation between serum and salivary cortisol concentration in horses has been reported [14,15]. Additional parameters for stress determination (used in various equestrian competitions) are heart rate (HR) and rectal temperature (TR) [2,13,16]. All these methods, however, require physical contact with the horse that could confound the measurement of stress. In exercise studies, a further limitation may arise in the inability of these methods to distinguish stressors originating from emotional and physiological (exercise) stimuli.

Infrared thermography (IRT) records the naturally emitted infrared radiation from the skin surface, providing a pictorial representation of body surface temperature [17]. Infrared thermography is already well established in equine medicine as a complementary diagnostic method for inflammatory conditions, vascular, and neurological disorders or the monitoring of physiological responses to changing environmental conditions [18–20]. Recently, an entirely noninvasive technique for measuring stress in horses has been developed, using IRT to measure the temperature from the area around the posterior border of the eyelid and the caruncula lacrimalis [10,21]. This area has rich capillary beds innervated by the sympathetic nervous system and thus represents an ideal place for measuring local changes in blood flow mediated by autonomic processes [22–24]. To date, however, there are no studies reporting the use of IRT as a noninvasive measure of stress in young racehorses in their first year of racing.

Johnson et al [25] have also reported a moderate correlation between maximum eye pixel temperature and TR in ponies, suggesting that eye temperature could be a simple and valid index as a first-line screen for fever in the equine. However, any two techniques measuring the same parameter in the same experimental subjects are likely to be closely correlated. A more rigorous assessment of the equivalence of two measurement techniques is provided by the agreement between the methods, as described by Bland and Altman [26] in their seminal article. To the best of our knowledge, there are as yet no studies reporting the agreement between maximum pixel temperature at the eye and core temperature in the equine, although in humans, Teunissen and Daanen [27] have cast doubt on the validity of eye temperature as an estimate of core temperature for fever detection.

Given the paucity of data published on eye temperature and stress indicators in young racehorses, the aim of our study was to investigate the agreement between maximum eye pixel temperature (as measured by IRT) and TR in this group and compare the temperature results with salivary cortisol concentration and HR both at rest and in response to exercise.

## 2. Materials and Methods

### 2.1. Study Population

Nineteen clinically healthy horses of two breeds (10 Thoroughbred [all 2 years old, 4 mares, 6 stallions] and

9 Arabian Horses [all 3 years old, 5 mares, 4 stallions]) with no stereotypic behavior were used in the study. All experimental procedures were approved by the Local Ethical Committee for Experiments on Animals in Wrocław. The study took place during August 2015 in the middle of the racing season. All horses were trained for flat racing at Partynice Race Course (Poland) during their first racing season. The examined horses were trained during the week at different intensity levels on the same racetrack by the same trainers throughout the whole training period and were housed in individual stalls with common management regimes. The daily routine during the study days was not varied. Horses were fed a standard diet for racehorses with typical fodder (hay and concentrate) three times daily at 5:30 AM, 11:30 AM, and 4:00 PM. They had ad libitum access to water.

All measurement techniques were used on each horse on the 3 days of intensive training scheduled in the training timetable (Tuesday, Friday, and the following Tuesday). On these days, horses underwent high-intensity racing training comprising warm-up for 10 minutes, then repeated canter at a distance of 2,200 m over half an hour. After training, they were untacked in the stable and cooled down on an automatic horse walker for approximately 20 minutes. On the days before each measurement, all the horses underwent light-intensity training comprising warm-up for 10 minutes, then repeated trot over 1,000 m and canter over 500 m for half an hour.

### 2.2. Infrared Thermography

Thermographic images were performed using a Variocam hr Resolution infrared camera (uncooled microbolometer focal plane array, resolution 640 × 480 pixels, spectral range 7.5–14 μm, InfraTec, Dresden, Germany). The protocol for thermography was as previously described by Valera et al [21]. To minimize the effect of environmental factors, thermography was always performed within an enclosed stable (in the horse's box). Since Hall et al [4] have demonstrated no significant difference between right and left eye temperatures, thermographic images of the left eye were collected three times per day:

- Between 6 AM and 7 AM (Fig. 1), when horses were at rest before training (BT),
- Between 7 AM and 9 AM (Fig. 2), within 5 minutes after the end of training (T+5), and
- Between 9 AM and 11 AM (Fig. 3), 2 hours after training when the horse was resting in the stable (T+120).

The distance of the animal from the camera was fixed for all imaging at 1 m, at an angle of 90° to the head, and the emissivity ( $\epsilon$ ) was set to 1 for all readings. Several images were performed per eye, allowing only the thermograms meeting the criteria for imaging distance and angle to be selected later for further analysis. In the case that more than one image met the protocol criteria, the mean of maximum eye temperatures was calculated. Based on the findings of Bartolomé et al [28], Cook et al [29], and Stewart et al [23], the maximum pixel temperature at the lacrimal

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