



An investigation into factors influencing basal eye temperature in the domestic horse (*Equus caballus*) when measured using infrared thermography in field conditions

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ABSTRACT

Infrared thermography (IRT) is a popular technology used for the detection of thermal changes given its non-invasive nature and lack of direct contact with the individual. Accordingly, the maximal eye temperature (MaxET) measured with IRT has been extensively applied in equine research. However, there is little information available about the potential limitations of the MaxET in field studies. Thus, the aims of this study were to 1) quantify the individual variation of MaxET in field conditions and the effects of individual, breed, body size (height at withers), eye side, sex and age, 2) determine the effects of environment and operator, and 3) explore the relationship between MaxET and rectal temperature (RT) at rest. To accomplish these aims, 791 MaxET measures from 32 horses were collected in Sweden in five different months and five farms over a period of 12 months. There was an effect of individual on IRT ($P < .05$) and individual MaxET varied from 29.4 to 37.6 °C. IRT was also affected ($P < .05$) by breed and sex (maximal difference 1.1 °C and 0.3 °C, respectively) but not by eye side, age and height at withers. There were significant effects of month and farm (maximal differences; 2.4 and 2.3 °C, respectively), between outdoor and indoor measurements (0.8 °C) and also between operators (0.2 °C). There were no correlations between MaxET and RT. These results demonstrate that in horses observed at rest in their home environment, MaxET is affected by endogenous (sex and breed) and environmental factors (farm, location and month of the year) and shows no relationship to RT. We strongly suggest that IRT technology should be used with great caution in field studies and only under conditions where these factors can be accurately accounted for.

1. Introduction

Body temperature is a critical homeostatic parameter for survival in mammals and a well-established key indicator of vital signs along with blood pressure, heart rate and respiratory rate [1]. The body's temperature is regulated by the thermoregulatory centre in the hypothalamus and is a result of cellular metabolism, the resulting heat production and the body's control of heat dissipation [2]. Although heat production varies amongst organs, the cardiovascular system provides efficient distribution of heat and even temperature of vital organs [2]. The hypothalamus receives input from warm and cold receptors in the skin, internal organs and the hypothalamus itself. Based on this information it can then alter behaviour and local tissue blood flow thereby influencing

local tissue temperature [2]. For example, in horses exercising in hot conditions, jugular vein temperature and rectal temperature are not the same [3], while in cold exposed horses, skin temperature and rectal temperature differ [4]. Heat that is dissipated through the skin can be monitored without contact and non-invasively using infrared thermography (IRT) [5]. As a result, this technology has the potential to provide new insights into a wide range of areas, from comparative thermo-physiological responses between captive and wild animals to injury and disease diagnosis [6].

Previous studies in different species also suggest that eye temperature measured with IRT can be used as a potential tool to study animal welfare [7]. It has been widely demonstrated that biological links exist between an individual's metabolism and stress levels [8,9]. Under

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potential stressors, the autonomic nervous system (ANS) and the hypothalamic-pituitary-adrenal (HPA) axis are activated, increasing blood flow as well as heart rate, catecholamine and cortisol levels. As a result of the increased peripheral blood flow due to sympathetic nervous system-mediated vasoconstriction and vasodilation, changes occur in an animal's heat production and loss. In the eye, there are small areas which have rich capillary beds innervated by the sympathetic nervous system. The medial posterior palpebral border of the lower eyelid and the lacrimal caruncle have been suggested as optimal locations for the detection of blood flow changes with IRT, in relation to ANS activity [7, 10].

Evidence of changes in eye temperature have been reported in response to adrenaline infusion in comparison to a saline-infused control in farm animals [10, 11]. A short report in horses also revealed a positive correlation between cortisol and maximal eye temperature after ACTH injection, although the experiment did not consider a control [12]. As a result, there has been increased interest in the use of IRT to investigate emotions and arousal from an animal welfare perspective [13]. In livestock species, eye temperature has also been used to detect stress or diseases but field studies indicate that eye temperature measured with IRT is highly disturbed by environmental factors. Many researchers recognize that high humidity, distance between the individual and the camera, angle, solar loadings and wind are major factors influencing IRT measurements [6, 14, 15, 16]. In horses, maximal eye temperature has been applied to detect body temperature/fever, stress from competitions, the fit of different bridles, aversive management (e.g. clipping), fear, housing system, physical exercise/fitness and poor performance [17, 18, 19, 20, 21, 22, 23, 24, 25, 26]. Ambient temperature, humidity, distance and angle are generally controlled in horse studies [27]. However, there is little knowledge about the effect of endogenous and environmental factors (e.g. sex, height at withers, month, light exposure) in equine field studies.

It is known that small animals generally have higher body temperature than larger animals and also that body temperature is affected by age and individual [28, 29, 30]. In horses, higher metabolic energy requirements for maintenance have been observed in individuals around 4 years of age compared to individuals around 11 years of age [31], indicating a higher metabolic rate in younger horses. This is also in line with the growth patterns, where growth is generally completed by 3 years of age [32]. Other sources of variation in metabolic rate and body tissue temperatures are body composition and tissue capillarisation. Body oxygen consumption (and thereby metabolic rate) is more closely related to lean body mass than to total body mass [33] and it is well known that body composition, energy requirements and body temperature differ between breeds of horses [34, 35, 36] as well as between horses with different levels of athletic fitness [37]. The energy requirements of stallions are estimated to be higher than for mares [32] and it was recently shown that their body temperature (registered through a chip implanted in the neckline) is also higher [36].

In humans, lateralized differences in cerebral blood flow exist, e.g. potentially causing differences in temperature of the left/right tympanic membrane [38]. These differences in ear temperature have been linked to stress in mammals [39, 40]. In horses, no differences in temperature between both eyes have been reported [41], although these measurements were collected in an outdoor arena and, thus, influenced by light exposure and other external environmental factors. Whether or not cerebral blood flow lateralization can be reflected in a lateralized temperature difference at the level of the eye has not been deeply investigated to date.

Although there are indications that eye temperature has a genetic component [42], more research is needed to assess how much of the proportion of the eye temperature is explained by the individual; in particular, for long-term monitoring studies under different environmental conditions. In cattle, at least 3 replicate images are needed to achieve high level of precision and variation between operators had a significant effect on maximal eye temperature, suggesting that a basic

standardized protocol might help to limit this variation in an agricultural environment [43, 44]. It is of interest to repeat an evaluation of a potential effect of operator since knowledge about this limitation might be crucial for the use of the equipment during field conditions. To our knowledge, there are no studies in horses investigating the effect of operator variability on eye temperature measured in field conditions. As such, the objectives of the present study were to: 1) quantify the basal individual variation of IRT eye temperature in field conditions and the effects of individual, breed, body size (height at withers), eye side, sex and age, 2) determine the effects of environment and operator, and 3) explore the relationship between eye temperature and rectal temperature (RT) at rest in the domestic horse (*Equus caballus*).

2. Material and methods

The study consisted of two experiments. In the first experiment the effect of different endogenous and environmental factors on the eye temperature were studied in 32 horses (8 young individuals were less than 4 years old and 24 adult individuals with ≥ 4 years old), in 5 farms, at five occasions (days) during 12 months, and in the second experiment the relationship between eye temperature and HR at rest was studied in 13 horses in two farms at one occasion.

2.1. Experiment 1

The first experiment was performed in Sweden south of Uppsala from June 2019 to May 2020 (Table 1). The eye temperature measurements were performed outside in daylight (from 3pm to 5pm) and indoors in the horses' usual / familiar stable (1–2 min between outdoor and stable measurements) by two camera operators in a random order and both the right and the left eye were measured, also in a random order. The outdoor measures were collected during cloudy days and the indoor measures, with only natural light and without cooling/heating system, within minutes either before or after (random) the outdoor measurements. Ambient temperature ($^{\circ}\text{C}$) and humidity (%) were registered with an electronic thermo-hygrometer IROX (Elfa Distrelec, Kista, Sweden). Information about the age (1.5–27 years old), sex (mare, stallion or gelding), breed and height at withers ($<$ or $>$ 148 cm) of the horses was registered (Table 2). All individuals were healthy according to the owners and kept on pasture or in paddocks with grass prior to the measurements.

The endogenous and environmental factor effects considered and the number of records processed are described in Table 2. Briefly, 791 infrared eye temperature measurements were recorded in a total of 9 ponies ($<$ 148 cm height at withers) belonging to three different breeds (4 mares, 4 geldings and 1 stallion, age 4–27 years) and 23 horses ($>$ 148 cm height at withers) belonging to two different breeds (10 mares, 6 geldings and 7 stallions, age 1–16 years). Each horse was measured six consecutive times (3 replicates in the right and 3 replicates in the left eye) for each operator in each situation, and only the optimal measures where the horse was positioned in a 90° angle to the sagittal plane were kept to further analysis. The minimum number of replicates per operator, horse and eye considered was 2; with an average of 5.3 replicates used. In total, 110 observations (from 6 individuals) were recorded in June 2019, 122 observations (from 9 individuals, of which 4 were also measured in June 2019) in October 2019, 133 observations (from 6 horses, of which 2 were also measured in October 2019) in February 2020, 257 observations (from 13 horses, of which 1 was also measured in October 2019) in April 2020, and 169 observations (from 9 horses, of which 2 were also measured in October 2019 and 2 were measured in February 2020) in May 2020. The measurements were collected at five different farms with a minimum of 2 horses in each farm, ≥ 2 breeds in each farm (with one exception where only Shetland ponies were measured) and 1 individual measured in two different farms (2 and 3). Measurements were made on at least two farms on every occasion (day), thus climate and light conditions were the same.

Table 1Description of the average (\pm standard error) ambient environmental parameters recorded during the five different months studied.

Month Location (indoors and outdoors)	Jun-19		Oct-19		Feb-20		Apr-20		May-20	
	In	Out	In	Out	In	Out	In	Out	In	Out
Ambient temperature (°C)	25 \pm 0 ^d	27 \pm 0 ^d	14 \pm 1 ^{bc}	12 \pm 1 ^{bc}	7 \pm 0 ^a	4 \pm 0 ^a	9 \pm 1 ^{ab}	5 \pm 0 ^{ab}	20 \pm 1 ^{cd}	18 \pm 1 ^{cd}
Humidity (%)	50 \pm 0 ^a	47 \pm 0 ^a	54 \pm 0 ^a	55 \pm 2 ^a	44 \pm 1 ^a	47 \pm 4 ^a	38 \pm 2 ^a	48 \pm 0 ^a	42 \pm 4 ^a	38 \pm 3 ^a
Atmospheric pressure (mbar) ¹		1009 \pm 0 ^c		999 \pm 1 ^a		1019 \pm 1 ^d		1006 \pm 0 ^b		1024 \pm 1 ^e
Wind speed (km/h) ¹		5 \pm 0 ^a		37 \pm 2 ^c		22 \pm 1 ^b		14 \pm 2 ^{ab}		17 \pm 2 ^{ab}
Wind direction ¹		WSW ²		SW ²		SW ²		NNE ²		NNE ²
Rain (mL) ¹		0		0		0		0		0

¹ Parameters retrieved from the Arlanda airport weather station (www.wunderground.com).² WSW is West-South-West, SW is South-West, NNE is North-North-East. Significant difference within groups (month and location) is identified with different letters in superscript (Tukey-adjusted comparisons; $p < .05$).**Table 2**

Description of the factor effects considered in the model and the number of individuals and records processed in the study.

Factors	Levels	N	
		Individuals	Records
Age	Young	8	153
	Adult	24	638
Eye side	Left	32	398
	Right	32	393
Height at withers	< 148 cm	9	302
	> 148 cm	23	489
Breed	Ponies ¹ (Gotlandsruss, Shetland, Welsh and Icelandic)	9	302
	Standardbred Trotter	8	155
	Swedish Warmblood	15	334
Sex	Mares	14	328
	Gelding	10	304
	Stallions	8	159
Operator	A	32	432
	B	32	359
Location	Indoors	30	367
	Outdoors	30	424
Month	June 2019	6	110
	October 2019	9	122
	February 2020	6	133
	April 2020	13	257
	May 2020	9	169
Farm	1	6	185
	2	10	276
	3	2	48
	4	10	191
	5	5	91

¹ Horses below 148 in height at withers.

2.2. Experiment 2

The second experiment consisted of a one-day test south of Uppsala (Sweden, July 2020), where eye temperature of the left eye [27] (6 replicates in total) and rectal temperature were measured at rest, within 3–4 min as described in Fig. 1.

The measurements were performed in the stable in two different farms from experiment 1 with only natural light and without cooling/heating system. The measurements were collected during morning (from 8am to 9am) in farm 1 and during afternoon (from 4pm to 6pm) in both farms. Ambient temperature was 18 °C and relative humidity was 64%. Seven Swedish Warmblood (mares, age 4–10 years; farm 1), one Icelandic (gelding, age 5 years; farm 1), two Shetlands (gelding and mare, age 8 and 11 years; farm 1), one Standardbred trotter (gelding, age 6 years; farm 2), and two Gotlandruss (gelding and mare, age 5 and 28 years; farm 2) were measured.

2.2.1. Rectal temperature

After the first thermographic examination, RT was taken by the same operator with a digital thermometer Geratherm© Rapid GT-195–1,

**Fig. 1.** Description of the sequential steps in experiment 2.

emitting an acoustic signal when the attained temperature remained stable for greater than 15 s.

2.3. Infrared thermography (IRT) measurements

All IRT images of the eye region (experiment 1 and 2) were taken with the camera positioned at a 90° angle to the sagittal plane and at a distance of approximately 1 m from the side of the animal with a frequently used FLIR i6 camera (FLIR Systems AB, FLIR Systems, Inc., Sweden). The resolution of the IRT camera is 160 × 120 pixels, with an operating environmental T range of –15 °C to 50 °C, a thermic sensitivity of <0.06 °C and an accuracy of \pm 2 °C or \pm 2% of reading. Ambient temperature and humidity are important required camera inputs in order to take accurate measurements according to the camera manufacturer, and were recorded either outside in the field or inside in the stables with an electronic thermo-hygrometer IROX (Elfa Distrelec, Kista, Sweden) (Table 1). The same image of a Lambert surface was taken every working session to define the radiance emission and to nullify the effect of surface reflections on tested animals [45]. Emissivity was set to 0.98 (mammal skin) [46], and IRT images were analysed using the FLIR Tools® software (FLIR Systems, Inc., Sweden). The maximal eye temperature (MaxET, °C) from the medial posterior palpebral border of the lower eyelid and the lacrimal caruncle in a circular area with a 1-cm diameter was recorded as described in Fig. 1.

2.4. Collection of weather data

Ambient environmental parameters by month are described in Table 1. Outdoor atmospheric pressure (mbar), wind speed (km/h), wind direction and rain (mL) were retrieved from the Arlanda airport weather station (www.wunderground.com) situated no more than 26 km from any of the farms. Ambient temperature (°C) and humidity (%) were collected both outdoors and indoors using the same equipment in

both experiments (IROX Elfa Distrelec, Sweden).

2.5. Statistical analysis

Mean differences between months and locations for the ambient environmental parameters recorded were tested using Tukey's HSD as a post hoc test. For experiment 1, intra-individual and inter-individual variations were examined by looking at the coefficient of variation (CV) using the following equation:

$$CV = (s.d./\mu) * 100$$

Where s.d. represents the standard deviation between each of the individual replicates (intra-individual) or between the complete dataset (inter-individual) and μ is the average between each of the individual replicates (intra-individual) or between the complete dataset (inter-individual).

A repeated measures linear mixed-effect model was used to examine the effect of the endogenous and environmental factors on the MaxET using the *lmer* function from 'lme4' package in R software [47]. The dependant variable (MaxET) was moderately negatively skewed and did not follow the normality (Shapiro-Wilk p-value = 1.797e-07), and therefore, had to be transformed using the formula "sqrt(max(MaxET+1) - MaxET)". However, non-transformed mean values for the MaxET were presented in the results section for easier interpretation. Breed, sex, operator, location of measurement (outdoors or indoors), month of measurement and farm were included as fixed effects in the final model, and the individual repeated measures were considered as a random effect. A probability level of $P < .05$ was chosen as the limit for statistical significance in all tests. The proportion of total variation apportioned to the individual was computed using the *lmerSummary* function from the 'VCA' package [48]. Other possible effects were also tested in an initial repeated measures linear mixed-effect model. The tested fixed effects were the age (young individuals with less than 4 years and adult individuals with ≥ 4 years), the side (right and left eye) or the height at withers ($<$ or $>$ 148 cm, called pony or horse, respectively). None of these effects were found to be significant ($P > .05$) and were thus not included in the final linear model.

For experiment 2, all measurements (MaxET and RT) were normally distributed ($P > .05$, Shapiro-Wilk). A two-tailed Student's *t*-test in R was first used to test differences in eye temperature measurements before and after RT measurement, and between morning and afternoon. No significant differences were observed ($P > .05$), and the average values for the MaxET measures (6 replicates in total) were then used for further analysis. The correlations between all measured parameters were calculated using the Pearson product-moment correlation coefficient, *r*.

3. Results

3.1. Individual variation of IRT eye temperature measurements

The description of the values obtained for the MaxET measurements in the medial posterior palpebral border of the lower eyelid and the lacrimal caruncle area (Fig. 2), are shown in Table 3. The mean value for the MaxET was 33.8 °C (ranging between 29.4 and 37.6; Table 3). In general, the majority of the individuals presented standard errors below 0.2 and variance values below 1.0. The intra-individual CV of the MaxET ranged between 0.6 and 2.6%, and the mean of the intra-individual CV (1.5%) was lower than the mean of the inter-individual CV (4.0%; Table 3). The linear mixed model analysis showed that there were individual differences on the MaxET measures ($P < .05$), and the proportion of total variation attributed to the individual was 37.4% (results not shown).

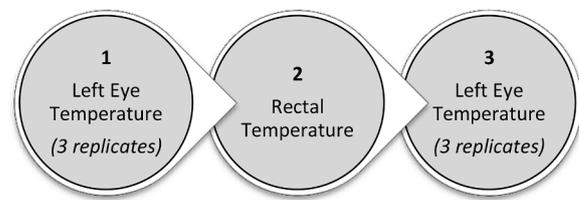


Fig. 2. Screenshot of an example of the right eye real image (a) and thermogram image (b) from the software *FLIR Tools version 5.13*. The images were captured with a 90° angle to the sagittal plane, at a distance of approximately 1 m, and emissivity of 0.98. The red triangle corresponds to the maximal temperature (°C), in the circular area (1-cm diameter) of the medial posterior palpebral border of the lower eyelid and the lacrimal caruncle.

3.2. Endogenous and environmental factors affecting IRT eye temperature measurements

The linear mixed model analysis showed that there were significant differences between breeds, sex, operator, location, month and farm for the MaxET ($P < .05$; Table 4). The MaxET from Standardbred trotters was significantly higher in comparison to the MaxET from the reference Swedish Warmblood horses ($P < .05$; Table 4), but ponies were not different from the reference ($P = .215$; Table 4). There were also significant differences between sexes, with MaxET from the stallions being higher than the MaxET from the reference mares ($P = .017$; Table 4), although geldings did not differ from the reference ($P = .264$; Table 4). The measurements collected were significantly different between operators ($P < .05$; Table 4). The measurements taken indoors were significantly higher in comparison to the measurements taken outdoors ($P < .05$; Table 4). The MaxET registered in the different months was significantly different in comparison to the reference June 2019 ($P < .05$; Table 4), although the MaxET registered in April 2020 did not differ from October 2019 or May 2020 ($P \geq .05$, results not shown). The measurements from farm 3 were significantly lower in comparison to the reference farm 2 ($P < .05$; Table 4), and the measurements from farm 4 were significantly higher in comparison to the reference farm 2 ($P < .05$; Table 4).

3.3. Relationship between eye temperature and rectal temperature

The average values for the MaxET and RT were 34.6 ± 0.1 °C and 37.4 ± 0.1 °C, respectively. There were no significant correlations between MaxET and RT ($P \geq .05$, Table 5).

4. Discussion

The results of the current study clearly show that individual, endogenous (sex and breed), and environmental conditions influence eye temperature. Consequently, the need for better control of such factors in future field studies using this technique is essential. Strict standardisation and control of several environmental parameters is highly recommended in future IRT field studies on horses. In this study, although MaxET differed between horses, less than 40% of the variation found was attributable to the individual when different environmental conditions were considered (e.g. month). In cows, experiments conducted in Ireland showed that between 49 and 80% of the variation was attributable to the individual for the maximal eye temperature; however, these experiments considered repeated measurements over a period of 1 or 6 months maximum [43, 44]. The lower percentage observed in the current study may be related to climatological differences between countries or to larger environmental effects on eye temperature measures over different seasons (e.g. Ireland experiments were conducted in autumn and winter but did not consider measurements in spring or summer seasons, when according to our results, the eye temperature increases more than 2 °C). However, the larger

Table 3
Description of the maximal¹ infrared eye temperature values obtained in the area studied.

Individual	Records	Mean	Std. Error	Minimum	Maximum	Variance	CV (%) ²
1	42	34.3	0.2	31.2	36.2	1.5	1.6
2	46	34.4	0.1	32.5	36.2	1.0	1.3
3	44	34.3	0.2	31.0	36.5	1.9	1.7
4	33	33.8	0.4	30.1	36.8	4.3	2.0
5	9	36.5	0.2	35.8	37.6	0.3	1.4
6	11	34.9	0.2	33.7	35.9	0.4	1.4
7	29	32.8	0.2	30.1	34.3	0.9	2.6
8	28	33.4	0.2	31.6	34.9	1.0	1.9
9	28	31.1	0.2	29.4	32.1	0.7	1.5
10	29	34.3	0.1	33.0	35.0	0.3	1.0
11	29	33.3	0.2	30.3	34.8	0.9	1.5
12	24	32.2	0.2	30.9	33.5	0.6	1.9
13	42	33.0	0.2	29.6	35.5	2.3	2.2
14	23	33.6	0.2	32.2	35.2	0.7	2.4
15	47	33.8	0.1	30.9	35.1	0.9	1.4
16	16	34.0	0.1	32.7	35.3	0.3	1.6
17	21	33.8	0.1	32.4	35.0	0.3	1.2
18	15	33.5	0.2	32.0	34.5	0.4	1.2
19	18	34.5	0.1	33.5	35.2	0.3	1.1
20	22	33.2	0.2	31.6	34.2	0.5	1.5
21	16	32.5	0.2	31.1	34.2	0.5	1.2
22	22	34.0	0.2	31.3	35.7	0.9	2.2
23	23	33.4	0.1	32.1	34.4	0.3	1.4
24	17	33.3	0.2	32.2	34.9	0.5	0.6
25	21	33.5	0.3	31.1	35.0	1.7	1.4
26	24	35.1	0.2	32.1	36.8	1.3	2.4
27	21	34.5	0.1	33.1	35.5	0.4	1.6
28	23	34.5	0.2	32.5	36.0	0.7	1.3
29	24	33.8	0.1	32.2	34.5	0.3	0.6
30	11	35.3	0.1	34.8	35.7	0.1	0.6
31	9	35.1	0.1	34.5	35.5	0.1	0.7
32	24	34.6	0.1	33.6	35.9	0.3	1.4
All	791	33.8	0.0	29.4	37.6	1.8	4.0

Std. Error = standard error.

¹ Maximal value from the circular area (1-cm diameter) of the medial posterior palpebral border of the lower eyelid and the lacrimal caruncle.

² CV = coefficient of variation was computed considering at least 2 replicates (average = 5.3 replicates per sampler and horse).

Table 4
Results of linear mixed model analysis of the maximal eye temperature (MaxET¹) in the medial posterior palpebral border of the lower eyelid and the lacrimal caruncle area. Bold p-values indicate a significant result (< 0.05).

Model: MaxET ² ~ Breed + Sex + Operator + Location + Month + Farm + Individual (random effect)				
Factors		Mean (°C)	SE	p-value
Breed	Swedish Warmblood	33.5	0.070	Ref
	Ponies	33.7	0.079	.215
	Standardbred	34.6	0.089	.031
Sex	Mare	33.7	0.079	Ref
	Gelding	33.7	0.080	.264
	Stallion	34.0	0.084	.017
Operator	A	33.7	0.065	Ref
	B	33.9	0.071	.008
Location	Outdoors	33.4	0.066	Ref
	Indoors	34.2	0.063	<0.001
Month	June 2019	35.0	0.066	Ref
	October 2019	33.2	0.131	<0.001
	February 2020	32.6	0.112	<0.001
	April 2020	33.7	0.101	<0.001
	May 2020	34.4	0.056	<0.001
Farm	2	33.5	0.081	Ref
	1	34.4	0.108	.290
	3	32.2	0.158	.008
	4	33.6	0.067	.029
	5	34.5	0.084	.148

¹ Non-transformed Means and SE.

² Transformed data “sqrt(max(maxET+1) - maxET)”.

inter-individual variation observed in comparison to the intra-individual variation is indicative that individuals have a greater impact on the IRT eye temperatures than the environmental factors

Table 5
Pearson product-moment correlation coefficients between MaxET and RT.

	RT
MaxET	r = -0.096 P = .745

Abbreviations: MaxET, maximal eye temperature; RT, rectal temperature.

present during the image capture [44]. Thus, the results indicate that the method is still able to detect individual differences in eye temperature, even in long-term field studies with repeated measures. Consequently, it also should be noted that this type of camera has an accuracy of ±2 °C which may not be the ideal to detect physiological changes. The collection of repeated measures to increase accuracy and an appropriate sample size is recommended for all future studies using IRT if different groups of horses are going to be compared. Theoretically, based on this study, an inter-individual variation of 4% (SD of 1.4) means that approximately 135 horses are needed if a significant (P < .05) difference in eye temperature of 0.3 °C is to be detected (physiological effects in the same range as the sex effect) and approximately 2 horses are needed if a significant difference in eye temperature of 2.3 °C is to be found (physiological effects in the same range as farm effects) [49].

Although the method quantifies individual variations, several endogenous and environmental factors influenced the basal eye temperature. Interestingly, the results from the current study showed statistical differences between sexes. Stallions had higher maximal eye temperatures than mares or geldings. Sex-specific differences in energy metabolism and endocrine functions are well known in mammals, and

sex hormones are suggested to play a role in thermogenesis [50, 51]. To our knowledge, this is the first study to report sex-specific differences in maximal eye temperature. Accordingly, groups and treatments should be balanced with respect to sex and absolute values from studies including only one gender must not be extrapolated to other genders. Similarly, horse breed has a significant effect and the same precautions as for sex are recommended. Standardbred trotters horses were higher in eye temperature compared to the Swedish warmblood horses or the ponies. In racehorses, increased tissue capillarization has been observed in response to intense training [52, 53]. Increased capillarization on the eye region studied may explain the higher eye temperature observed in Standardbred trotters horses in comparison to other breeds. However, as most of the Standardbred trotters horses included in the study were in race training it is also likely that they had higher metabolic rate and heat production than the other breeds that were used for leisure riding or no activity at all.

Although age and body size are known to affect body temperature and thermoregulation in mammals [28, 29], our results on eye temperature were unable to support this. It is likely that the variation in height at withers was too small in our study (there is also no earlier suggestion that body temperature would differ between ponies and horses). A previous study in Standardbred trotters reported higher eye temperature at rest in young horses (2 to 3 years old) in comparison to adult horses (> 3 years old) [24]. The lack of influence of age in our study may also be related to the fact that the young horses in our study were older than 12 months when the most rapid growth phase is already completed [32], or due to limited sample size across breeds. In agreement with previous publications, no differences in the temperatures between the right and left eyes were detected [41]. The results indicate that there is not lateralization in eye temperature indicative of cerebral blood flow differences between left and right hemispheres.

Despite ambient air temperature and humidity having been entered into the camera's settings when eye temperature was measured, a high influence of other factors such as indoor vs outdoor measurements and month of the year were still detected. These results are in line with previous studies which have reported higher eye temperatures with sunlight exposure or warmer weather conditions [15, 16]. Outdoor measurements where wind and solar loading cannot be properly controlled should be avoided. However, the cause of these variations needs further investigation. In our study, months with similar ambient temperatures (e.g. June 2019 and May 2020), showed differences in MaxET. In addition, in months with great differences in ambient temperature (April vs May) there were no differences in eye temperature. Thus, other environmental parameters such as distance to surrounding objects emitting and interfering with radiation and maybe atmospheric pressure might have impact the MaxET [54, 55]. In future field studies, strict standardisation or at least control of environmental factors is recommended. Similarly, the effect of month is substantial and also in the same range as pathological changes. Observations made on groups at different months/seasons can therefore not be recommended. The local environment (eg. ambient temperature, humidity, atmospheric pressure, surrounding objects) is of importance for the results and registrations should be made in the same spot and position in all individuals and treatments.

The infrared radiation from the eye is likely to be affected by the changes in size of the pupil in relation to the light exposure. In mammals, the pupil dilates when the iris contracts and narrows when the iris is relaxed. This occurs in response to increased activity in the sympathetic and parasympathetic nerve fibres, respectively [56]. The size of the pupil also changes due to variations in light exposure and this is regulated by reflex action and light-sensitive sensory cells in the retina [56, 57]. In this study, eye temperature measurements were compared between outdoor and indoor conditions, resulting in an increase in eye temperature under conditions of reduced natural light (indoors). This increase in eye temperature might reflect the increase in pupil size mediated by the ANS, although more research with standardised

conditions is needed to corroborate this hypothesis. Future studies comparing the effects of different indoor artificial lighting systems could be very valuable to determine if there are changes in eye temperature associated with light exposure in horses.

Farm was a factor that influenced eye temperature measures. All surfaces with higher temperature than 0 K (-273 °C) emit radiation and surfaces with large differences in temperature from the object measured can disturb the IR registration [54]. During field and stable conditions, there are probably many options for interference. However, also endogenous factors might have influenced the results. Feeding level as well as type of diet affect heat loss from the body [58] and differences in feed intake between farms might, explain part of the differences in eye temperature observed between them. Despite this, the farm effect is substantial and in the same range as pathological changes (fever) on an individual level. This means that comparisons between farms, within the physiological range, cannot be recommended. In horses, no differences in eye temperature were observed between handlers of horses [59], but the operator effect has not been previously investigated. In cows, the operator had an effect on eye temperature [43]. During field conditions some variation can be expected, e.g. keeping exact 1 m distance between the camera and the eye. It is important, therefore, that thermal images are collected with standard operating procedures to minimize the variation between operators (e.g. operator handedness might contribute to the difference eye variation) [44]. In this study, both operators were right-handed, although a lack of training or substantial differences in height might have affected the angle and thus the eye temperature [27]. This study in horses shows that there may be an operator effect and therefore operator should be included in the statistical model if there are several operators (or only one operator shall be used in the same study).

There was no linear correlation between IR eye temperature and rectal temperature (measured using conduction). This indicates that eye temperature does not appear to be a sensitive method to monitor for example fever, where rectal temperature is traditionally used. This is however, not surprising since the accuracy of the IR camera, according to the manufacturer, is 2 °C. However, it is also possible that a non-linear behaviour between eye temperature and rectal temperature exists.

4. Conclusions

The results of this study showed that there were individual differences in the basal maximal eye temperature measured with IRT in horses during field conditions. Height at withers of the horse (e.g. pony vs horse) and age did not affect eye temperature measurements but endogenous (sex and breed) and environmental variation between months were major factors influencing eye temperature, and should be considered in the modelling and design of future field experiments. Strict standardisation or at least control of environmental parameters is highly recommended. No linear relationship was observed between maximal eye temperature and rectal temperature at rest, indicating that eye temperature is likely not suited to test fever.

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