General Articles

Reliability and repeatability of thermographic examination and the normal thermographic image of the thoracolumbar region in the horse

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Summary

Reasons for performing study: **Thermographic imaging is an increasingly used diagnostic tool. When performing thermography, guidelines suggest that horses should be left for 10–20 mins to 'acclimatise' to the thermographic imaging environment, with no experimental data to substantiate this recommendation. In addition, little objective work has been published on the repeatability and reliability of the data obtained. Thermography has been widely used to identify areas of abnormal body surface temperature in horses with back pathology; however, no normal data is available on the thermographic 'map' of the thoracolumbar region with which to compare horses with suspected pathology.**

Objectives: **To i) investigate whether equilibration of the thermographic subject was required and, if so, how long it should take, ii) investigate what factors affect time to equilibration, iii) investigate the repeatability and reliability of the technique and iv) generate a topographic thermographic 'map' of the thoracolumbar region.**

Methods: **A total of 52 horses were used. The following investigations were undertaken: thermal imaging validation, i.e. detection of movement around the baseline of an object of constant temperature; factors affecting equilibration; pattern reproducibility during equilibration and over time (n = 25); and imaging of the thoracolumbar region (n = 27).**

Results: **A 1°C change was detected in an object of stable temperature using this detection system, i.e the 'noise' in the system. The average time taken to equilibrate, i.e. reach a plateau temperature, was 39 mins (40.2 in the gluteal region, 36.2 in lateral thoracic region and 40.4 in metacarpophalangeal region). Only 19% of horses reached plateau within 10–20 mins. Of the factors analysed hair length and difference between the external environment and the internal environment where the measurements were being taken both significantly affected time to plateau (P<0.05). However, during equilibration, the thermographic patterns obtained did not change, nor when assessed over a 7 day period. A 'normal' map of the surface temperature of the thoracolumbar region has been produced, demonstrating that the midline is the hottest, with a fall off of 3°C either side of the midline.**

Conclusions: **This study demonstrates that horses may not need time to equilibrate prior to taking thermographic images and that thermographic patterns are reproducible over periods up to 7 days. A topographical thermographic 'map' of the thoracolumbar region has been obtained.**

Potential relevance: **Clinicians can obtain relevant thermographic images without the need for prior equilibration and can compare cases with thoracolumbar pathology to a normal topographic thermographic map.**

Introduction

Infrared thermography relates directly to the surface temperature of an object. Injured and diseased tissue has an altered temperature due to a change in blood flow and this may be viewed on a thermal image of the body, allowing anatomical location of the abnormality.

The use of thermography is becoming increasingly accepted in equine diagnostic imaging. Increases in body surface temperature ('hot spots') are related to increased blood flow associated with increased metabolic activity and an altered local circulation (Love 1980; Ring 1990). Clinically, these are usually associated with inflammation (Palmer 1980; Eddy *et al.* 2001), secondary to injury (Waldsmith and Oltmann 1994). Decreases in body surface temperature ('cold spots') are a result of reduced tissue perfusion, due to either vascular shunts, thrombosis or infarction (Turner 1991), or autonomic nervous system abnormalities (von Schweinitz 1999; Eddy *et al.* 2001).

Delahanty and Georgi (1965) first documented the use of thermal imaging in equine veterinary medicine and based its usage on thermal imaging techniques in the human body. For animals, when compared to man, the temperature of the surface

seen by the camera is usually not that of the skin but of some layer within the hair coat and a number of factors have been suggested to affect interpretation of the thermographic image. Local differences in skin temperature of animals with coats are attenuated at the surface to an extent, the degree depending on the thickness and nature of the coat (Clark and Cena 1977). Hair length has also been implicated in causing difficulties in interpretation of thermographic data, but clipping did not change the overall pattern in the limbs (Turner *et al.* 1983) and, contrary to the suggestion of Clark and Cena (1977), clipping was therefore not considered necessary to produce a reliable thermogram. However, it is suggested that the hair be short, of uniform length and lying flat against the skin to permit thermal conduction (Turner *et al.* 1983).

Turner *et al.* (1998) reviewed the use of thermography in equine medicine, also including previous studies in the human field (Barnes 1963; Delahanty and Georgi 1965; Clark and Cena 1977; Palmer 1981; Turner *et al.* 1983). From these studies, the various factors that need to be considered for the production of reliable thermograms in the horse were identified. However, to date there has been little validation of the repeatability and reliability of the technique, leading to questions as to its validity (Head and Dyson 2001).

From a clinical perspective, there are a number of reports detailing the thermal pattern in the limbs of the horse. Purohit and McCoy (1980), Vaden *et al.* (1980) and Palmer (1981) reported that all horses had a similar infrared emission pattern, which was affected by ambient temperature, although no 2 horses had exactly the same pattern (Purohit and McCoy 1980). The pattern obtained had a high degree of left to right symmetry (Purohit and McCoy 1980; Ring 1990), although asymmetry is not always considered abnormal (Kold and Chappell 1998). There was also a high degree of symmetry between the thoracic and pelvic limbs distal to the carpus and tarsus (Purohit and McCoy 1980). The detailed thermogram of the distal limb has been described in horses at rest and after exercise (Purohit and McCoy 1980; Palmer 1981; Turner *et al.* 1983, 1986). Thermography is often also used in the diagnosis of equine back pathology (Turner 1991; Colles *et al.* 1995; Ahern 1996; von Schweinitz 1999) and is currently the only diagnostic tool used in the diagnosis of a reflex sympathetic dystrophy-like syndrome in this region (von Schweinitz 1999). However, while a number of reports have described the thermographic appearance of the 'normal' equine back, no objective studies have been published.

The aims of this study were 2-fold: firstly to determine i) the time required for a horse to equilibrate fully to its environment and identify factors affecting this, ii) the effect of the equilibration process on the thermal pattern emitted and iii) whether the thermal image is reproducible over hourly, daily and weekly intervals; and secondly to identify whether a consistent thermal pattern exists in the thoracolumbar region of the horse, compare this pattern in ridden and unridden animals and create a quantitative map.

Materials and methods

Equipment

The thermal images were taken with a ThermaCAM PM280[1], a hand-held infrared imaging radiometer which has a palm-sized focal plane array (FPA) radiometer with full screen temperature measurement and built-in storage and analysis capabilities. It is TV compatible and was connected to a Sony PVM-14NIE Trinitron colour video monitor[2] for extensive real-time data analysis. The data, stored on removable solid-state SRAM PCMCIA memory cards as digital images, were copied to a PC running on Windows NT via an OmniDrive Professional SNr: 4079 - OmniCE[3] and analysed.

Analysis

Using the TherMonitor PRO software programme[4], lines were superimposed on saved thermographic images at anatomical sites as detailed below. The temperatures along the superimposed line were calculated by the software and plotted as temperature/distance graphs. Data from individual distance points were entered manually into a Microsoft Excel data handling programme and statistical analysis performed within this package.

Animals

The 25 horses used in *Part 1* of this study were either clinical cases referred for nonorthopaedic problems (n = 12), presented to the Queens' Veterinary Hospital Cambridge, or horses already resident on the university farm (n = 13). The 27 horses used in *Part 2* were 6 regularly ridden clinically normal mares (age 4–7 years) resident on the university farm (which had been used in *Part 1* of the study for other measurements) and 20 unridden (unbroken) mares (age 4–7 years) at the Equine Fertility Unit, Newmarket. All horses were assessed as normal on the basis of a clinical examination of back mobility and flexibility, history (where ridden), and each animal had ultrasonography of the supraspinous ligament, dorsal sacroiliac ligament and *longissimus dorsi* muscle performed after thermographic imaging in order to identify any soft tissue disease.

Preparation for measurements

For at least 1 h before acquisition of images: 1) field-kept horses were brought indoors; 2) rugs or supporting bandages were removed; 3) where appropriate, horses were groomed to remove mud and debris from the coat; 4) horses were thoroughly dried from any rain or sweat; and 5) horses were kept indoors, out of sunlight.

Thermal imaging validation

In order to evaluate the degree of temperature variation, a thermostatically controlled, equilibrated body was in the thermal imaging room when measured by the ThermaCAM, in the form of a water-bath with an electronically controlled thermostat set to 30°C, allowed to equilibrate within the thermal imaging room for 2 h. The subsurface temperature of the water was then measured every 3 mins for 1 h using both a mercury thermometer and the ThermaCAM following agitation of the water to ensure thermal mixing. For ThermaCAM imaging, the camera was positioned at exactly 40 cm from the water surface. Temperature changes over time were plotted for both sets of data and the degree of temperature wavering of the water was determined (Tunley 2002).

Factors affecting equilibration

Horses were taken individually from their original environment of known temperature, (measured using a preplaced mercury thermometer 1.5 m above ground level) and restrained in stocks in the thermal imaging room. Thermal images of the horses were
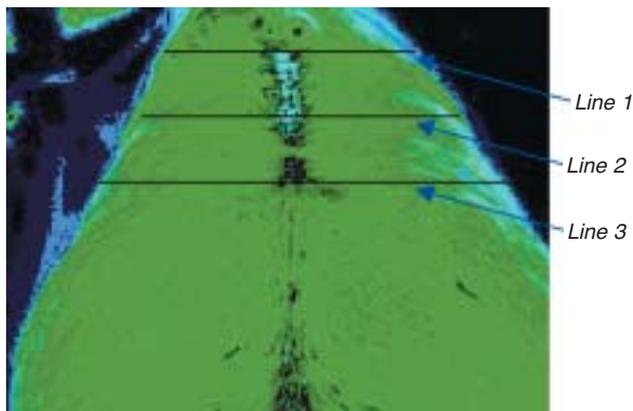
Fig 1: Thermal image of the back of a horse, dorsal view. The 3 horizontal black lines represent lines 1, 2 and 3, along which the temperatures were measured.
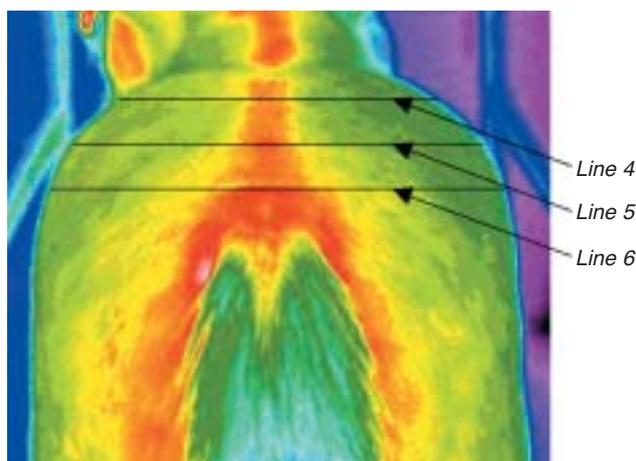

Fig 2: Thermal image of the gluteal area of a horse, dorsal view. The 3 horizontal black lines represent lines 4, 5 and 6, along which the temperatures were measured.

obtained at 3 min intervals for 1 h with a camera-horse distance of exactly 2 m. Point temperature was measured at the a) lateral metacarpophalangeal joint (centred on the distal metacarpus), b) lateral thorax (mid-point of body in a dorsoventral plane, equidistant between point of shoulder and *tuber coxae*) and c) gluteals (mid-point between *tuber coxae* and tail head). These anatomical sites were readily identifiable in thermographic images and easy to repeat between horses and in the same individual at different time measurement points.

The temperature at 1.5 m above ground level and humidity of the imaging room were recorded every 3 mins for the 1 h acclimatisation period using a freestanding mercury thermometer and linked hygrometer (Hygromat)[5].

The rectal temperature of the horse at the beginning, middle and end of the acclimatising period was measured using a digital

TABLE 1: Time taken for surface temperature to equilibrate

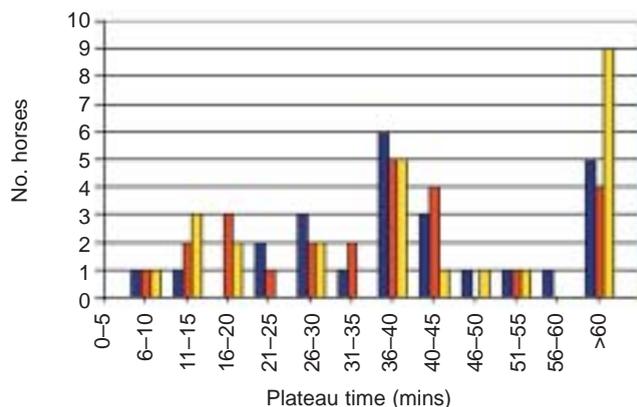| Body area | % reaching plateau | | |
	<20 mins	21–38 mins	>39 mins
Lateral thorax	8	28	64
Gluteal	24	20	56
Fetlock	24	28	48
Total	19	25	56

Fig 3: Time taken for horses to reach plateau temperature within specific time periods for each of the body areas measured. ■ = Lateral thorax; ■ = Gluteal area; ■ = Fetlock area.

thermometer. Average temperature changes over time were calculated for each of the 3 areas. Values were plotted on temperature/time graphs. The time taken for each area of the horses to reach plateau temperature was determined.

Factors measured on each horse included: a) bodyweight (kg), using an Olympian 2000 Equine Weigher[6]; b) height; c) colour, categorising subjectively into dark, medium and light hair; d) hair length (mm) - a hair pluck from the mid-thorax and mid-cannon (leg) regions was sellotaped to paper and hair length measured with a ruler; e) cannon circumference (cm), measured using a tape measure 1 cm below the carpus.

Pattern reproducibility during equilibration

In order to deduce the reliability of the pattern produced before, during and after equilibration, images were taken from the equilibrating horses at the beginning and end of the period and assessed objectively. Using the software package, the temperatures along a line drawn between 2 specific points (antebrachial fossa to point of withers) on each image were plotted on a temperature/ distance graph and the graphs generated at each time for each horse were compared. In order to standardise the graphs between different horses, the lines were resized to the same length. This was achieved by averaging groups of points together to obtain 101 points (0–100 inclusive), before plotting them and finding the average differences between the lines. The average difference in temperature between the 2 lines, i.e. a line taken from an image at the start of acclimatisation and an image at the end, was then subtracted from the end line. This was then replotted to give the 'end corrected' line.

TABLE 2: Significance (P values) of individual factors and their effect on the time taken to reach plateau temperatures using a regression analysis statistical test. Values in bold shows significance at the 95% confidence limit

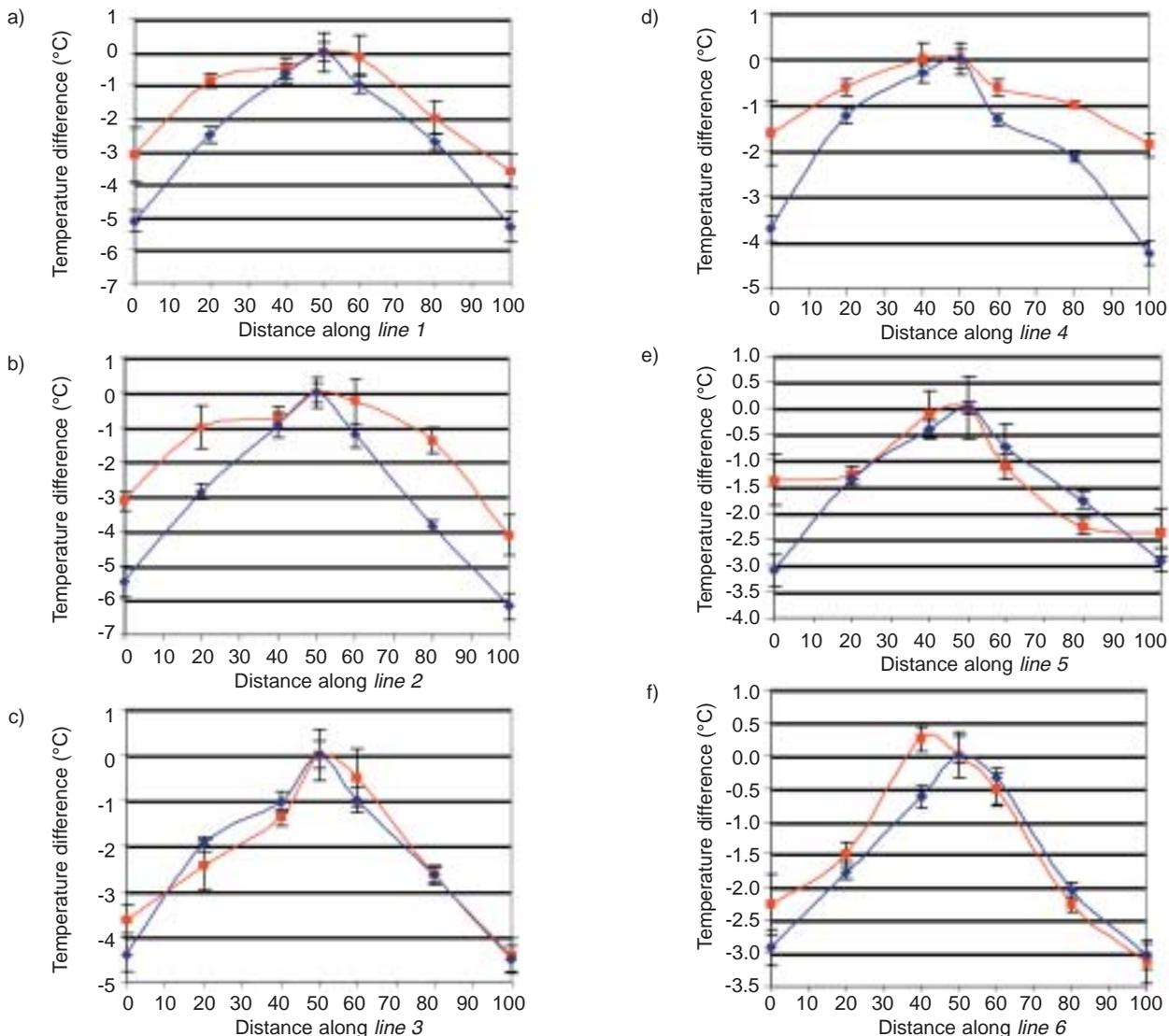| Factor	Lateral thorax	Gluteals	MCP joint
Humidity	0.174	0.053	0.394
Height	0.274	0.504	0.600
Temperature difference	**0.026**	**0.006**	0.161
Hair length	**0.003**	**0.026**	0.703
Colour	0.493	0.818	0.591
Weight	0.594	0.647	0.212
Cannon circumference	0.890	0.672	0.055

Fig 4: Mean ± s.d. across line 1 for temperature differences for ridden (■) and unbroken (♦) horses for lines a) 1, b) 2, c) 3, d) 4, e) 5 and f) 6.
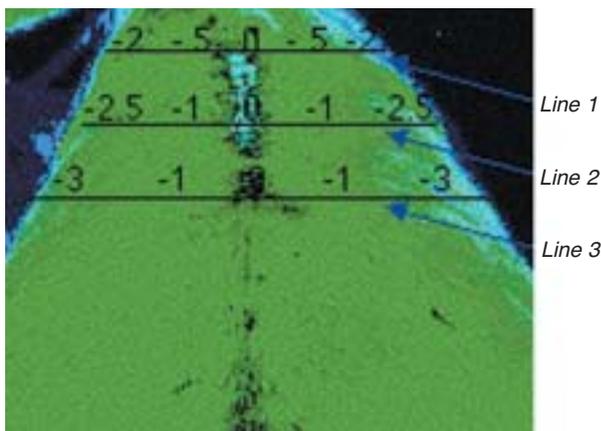

Fig 5: Normal reference range of temperatures for the back of the ridden horse. Midline = 0.

Fig 6: Normal reference range of temperature of the gluteals of the ridden horse. Midline temperature = 0.

Pattern reproducibility over time

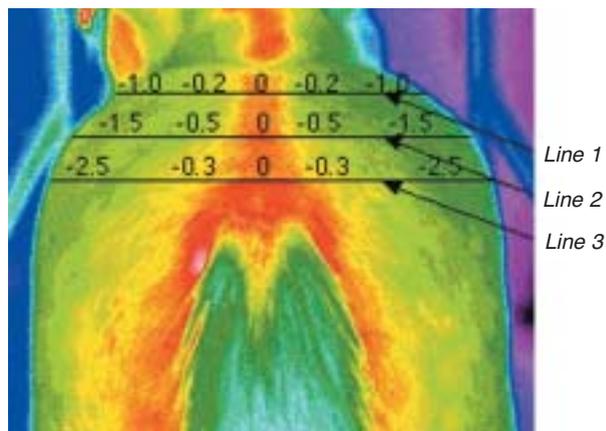The reproducibility of the thermal pattern was evaluated by imaging the lateral thorax and gluteal areas of 5 horses at intervals of 5 mins, 5 h, 2 days and 1 week. Again, the images were assessed objectively as described above. The objective assessment used lines reduced to the same length as described above to compare them to the first image obtained. The anatomical points

used in this experiment were the lateral thorax and gluteal regions as described above.

Thoracolumbar imaging

The thoracolumbar region of the back was imaged as described. After image acquisition, using the TherMonitorPRO software package[4], 6 lines were placed horizontally across the images at specific anatomical sites (Figs 1, 2). *Line 1* was placed at T9 (base of mane region), *line 2* at T12 (base of withers region), *line 3* at the same distance from *line 2* as *line 2* was from *line 1* (T15 region). In the gluteal region *line 4* was placed over S1, *line 5* between *lines 4* and *6* and *line 6* over the sacrococcygeal space region.

The temperatures along these lines were plotted on temperature/distance graphs. Mean ± s.d. temperature variations for both ridden and unbroken groups were deduced and paired *t* test analyses were performed on positions 0, 20, 40, 60, 80 and 100 for each of the lines, to compare the pattern of the ridden and unridden horses, i.e. deduce the significance of any differences between the 2 groups. Once this was established, the expected temperature variations across each line measured was annotated onto a thermographic image of the horse, the base line being the midline measured as zero, and the other measurements the expected difference in °C from the midline.

Results

Thermal imaging validation

Using a mercury thermometer, it was demonstrated that the surface temperature of the water bath was kept constant to within 0.1°C (29.1–29.2°C) throughout the test period. Using the ThermoCAM, it was demonstrated that the readings were not equal to the mercury thermometer readings but that they fluctuated within ± 0.5°C of the surface temperature as measured by the mercury thermometer, i.e. a 1°C change detected in a thermographically constant subject. The fact that this ThermaCAM detection system had an apparent 'noise' was used in interpretation of other experiments.

Factors affecting equilibration

The average time taken to reach plateau temperature for all body areas measured was 39 mins; gluteal average time was 40.2 mins, lateral thorax 36.2 mins and lateral aspect of the MCP joint 40.4 mins. One third of horses measured did not reach plateau temperature within 1 h. The percentages of horses reaching plateau temperature within various times for each of the body areas measured are shown in Table 1. The actual number of horses reaching plateau temperature within time periods for each of the body areas measured is illustrated in Figure 3.

Each of the factors measured and their effect on the time taken to reach plateau temperature were analysed using single variable regression analysis (Table 2), before the significant factors were analysed together using multivariate regression.

The factors with P value <0.05 (i.e. hair length and temperature difference) were then analysed together using multivariate regression to determine their combined effect on the time taken to reach plateau temperature. In the lateral thoracic region, the combined P value was 0.002 and in the gluteal region P = 0.012 (significant at 95% confidence). In the MCP region P = 0.392 (not significant at 95% confidence)

Pattern reproducibility during equilibration

The s.d. of differences between beginning and end 'corrected' lines was evaluated with a 95% confidence limit. Twenty-four of 25 horses had 95% confidence limit values within the allowed 'background noise' of 1°C, indicating that no significant pattern changes were occurring within the equilibration period in these horses. The one horse with significant change over this period suffered marked distress and excessive sweating over the measurement time period in response to being restrained in stocks.

Pattern reproducibility over time

Measurements were made in both anatomical regions at Days 0, 2, 5 and 7 in 5 horses. Analysis of raw data showed that individual lines in some of the graphs had some changes in pattern, but that the pattern was the 'same', defined by being within the background allowed noise of 1°C, with a 95% confidence limit in 5/5 horses at Day 2 and 4/5 horses at Days 5 and 7. All were within a 90% confidence limit at all time points.

Thoracolumbar measurements

The temperature of each horse at positions 0, 20, 40, 50, 60, 80 and 100 on each line were extrapolated from the graphs. The calculated mean and s.d. of the temperature gradients for all of the horses, both ridden and unridden (unbroken), for each of the 6 lines were then plotted on temperature/distance graphs (Figs 4a–f).

Statistical analysis

The paired *t* test analysis on all 6 lines demonstrated no significant differences between the 2 groups of horses (95% confidence) except at position 80 on *line 2* (P = 0.04).

Generation of 'normal' thermographic pattern

Using the data indicated above, the thermographic pattern in normal horses was illustrated and is shown in Figures 5 and 6.

Discussion

A number of authors have recommended that thermographic image acquisition should be performed after equilibration in order that images are acquired in a known external environment to facilitate interpretation (Barnes 1963; Keele *et al.* 1982; Turner *et al.* 1983). The first part of this study shows that, for all 3 sites studied, only 19% of sites acclimatised within the previously recommended time scale of 10–20 mins (Barnes 1963; Turner 1991). Twenty-five percent of horses equilibrated within 21–38 mins and 56% took greater than 39 mins.

A number of factors have been reported to affect the quality and interpretation of the thermographic image. In this study, we investigated whether size of horse (quantified in terms of bodyweight, height and cannon circumference), colour, hair length or temperature difference between the horses' original environment and thermal imaging room affected the time taken for the horses to equilibrate.

Using a 95% confidence limit, none of the factors had a significant effect on the time taken, apart from the temperature difference between the horses' original environment and the

thermal imaging room (temperature difference), and hair length. When analysed together they significantly affected the time taken for the horses to reach plateau temperature in the lateral thorax and gluteal areas, but had no significant effect on the time taken to reach plateau temperature in the MCP region. The contributing individual factors in the significance for the lateral thorax and the gluteal areas were different, i.e. the temperature difference was the significant factor in the gluteal area and hair length for the lateral thorax.

The mean values for equilibration time and role of hair length and temperature difference in the equilibration process imply that, in order for a horse to acclimatise to the surrounding room temperature, a recommended period of 39–60 mins should elapse before obtaining images, with 'hairy' horses needing longer acclimatisation times than clipped/short haired ones.

The second aim of our study was to investigate whether equilibration to the environment is actually required. In order to achieve this, the thermographic patterns in the shoulder region were converted to point measurements along a line drawn between the antebrachial joint and withers. These point measurements were then compared at the start of the equilibration period and the end (1 h later). Since 24/25 horses had no significant change in thermographic pattern during the 1 h of measurement, there appears to be little need for equilibration, as it is the thermographic pattern generated which is of diagnostic value (Hall *et al.* 1987).

However, while equilibration may not change the thermographic patterns produced, the process may enhance interpretation of images. Isolated areas of thermal changes due to pathology can cause both thermal hotspots and cold spots, the actual temperature of which is independent of normal thermoregulation (Love 1980; Ring 1990; Turner 1991; Waldsmith and Oltmann 1994). Given that visual pattern recognition depends on contrast between areas within an image, a pathological area causing a thermal hotspot (Delahanty and Georgi 1965; Stromberg 1973; Palmer 1980; Eddy *et al.* 2001) may therefore be more evident at the lower, pre-equilibration temperatures, as the temperature difference between normal and abnormal areas would be greater and the converse would be true for thermal cold spots. This is only true, however, when the horse is brought from the outside, ambient temperature into a warmer inside environment for imaging, as occurred in this study.

The use of thermography in a clinical setting usually includes taking repeat thermographic images of a horse, for example when monitoring a disease process. This study sought to evaluate the reproducibility of the thermal pattern of a horse over hourly, daily and weekly intervals. At the 95% confidence limit, 13/15 time points were not significantly different at the 90% confidence limit no time points were significantly different. These results indicate that, over a one week period, the majority of images did not differ, confirming that an individual horse does have a thermographically reproducible pattern from day to day in the lateral thoracic and gluteal regions.

This second part of this study described the thermographic appearance of the thoracolumbar region of normal ridden and unridden horses. The body surface temperature was quantified upon 6 lines superimposed on thermographic images in the mid-thoracic and gluteal region. The temperatures along these lines were compared graphically and statistically between ridden and unridden horses. In Figures 4a, b and c, the ridden horse lines appear to be flatter curves but, for Figures 4d, e and f, there is better visual agreement between both ridden and unridden patterns. Statistically, there was no significant difference between the profiles generated by the 2 groups of horses for any of the lines along the horses' backs, except at point 80 in *line 2* (P = 0.04), for which no obvious physiological explanation exists. The significance of this close agreement between the 2 groups of horses is that riding appears to have no bearing on the thermal image obtained in clinically normal animals, i.e. normal values and reference ranges remain the same for the 2 groups of animals.

All graphs show a higher temperature along the midline of the spine with a lowering temperature when moving abaxially. This is seen in both ridden and unridden animals, probably due to the high number of superficial subcuticular blood vessels found in this position (Ghoshal and Nand 1975) and reported previously (von Schweinitz 1999). In a study by Turner (1991) and in unpublished observations (C. Colles, personal communication), the midline is generally warmer, i.e. over the back, chest, between the hind legs and on the ventral midline. The warm stripe seen over the back has been reported to have fairly linear borders except at the lumbosacral junction, where it has flared borders. Other studies have reported that the trunk and regions are within 2°C of the midline reference and are symmetrical (von Schweinitz 1999). In our study, however, the temperatures away from the midline differed by up to 3°C, indicating that the range of temperatures seen within a normal image are wider than those previously reported.

In conclusion, the recommended period required for a horse to equilibrate fully to its environment is between 39 and 60 mins. The major factors affecting this equilibration time are the temperature difference between the original environment and that in which the images are obtained, and the length of hair on the horse. In practice, the easiest method of reducing the equilibration time is to reduce the temperature difference between the horse's environment and the thermographic examination area. Imaging a clipped horse would not affect the overall pattern of the image, but would reduce the time taken for the horse to equilibrate. Equilibration, however, did not affect the thermal pattern generated in 24/25 horses and may therefore not be necessary. We have also demonstrated that the thermal pattern generated by horses is reproducible over intervals of up to 1 week with a confidence limit of 90%. In the second part of this study, we have provided quantitative data on the normal thermographic pattern and values expected in the thoracolumbar region of a normal horses, whether ridden or unridden, and this should facilitate the interpretation of future studies on pathology in this region.

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